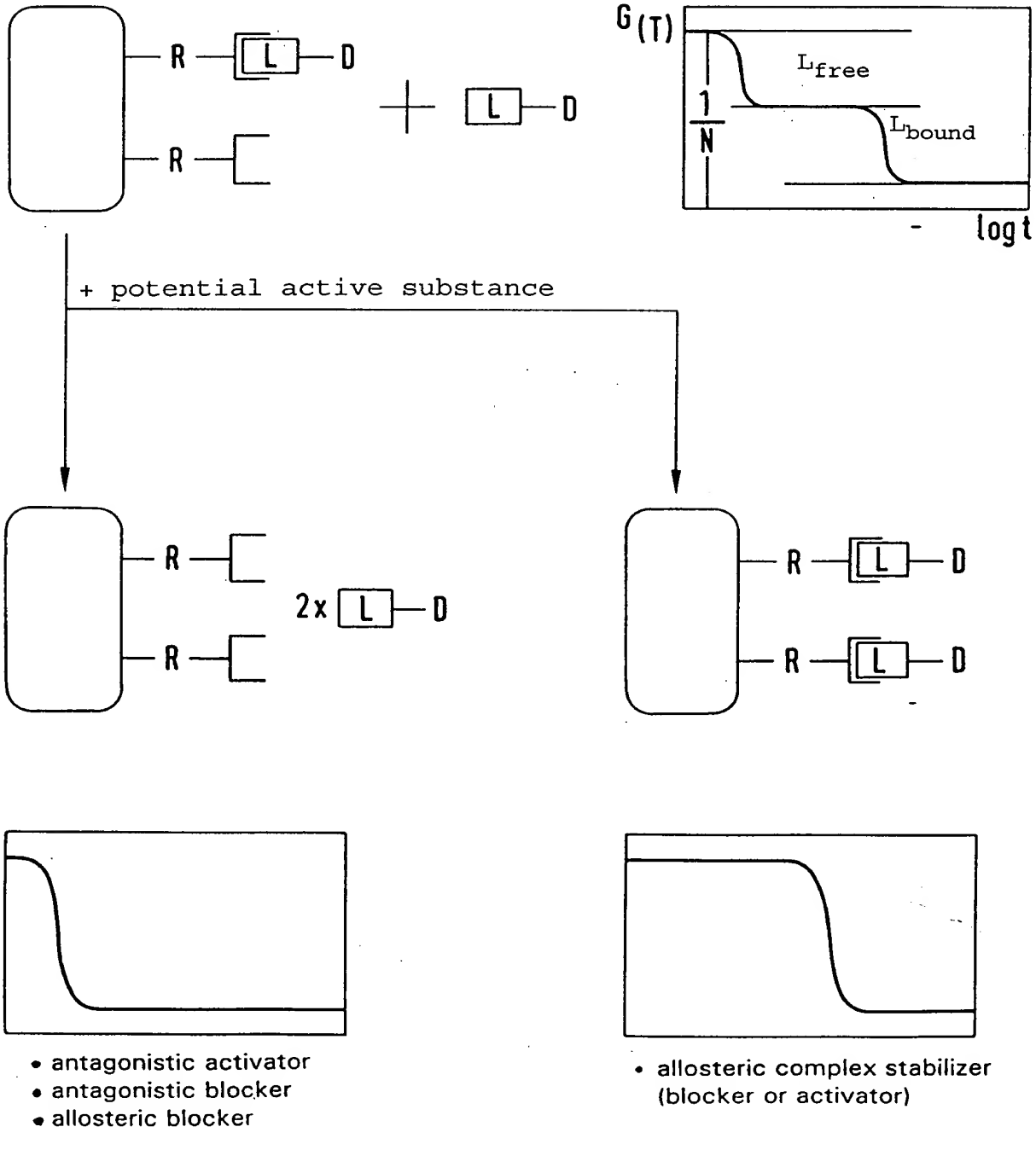
Receptor Assay (1)



- antagonistic activator
- antagonistic blocker
- allosteric blocker

- allosteric complex stabilizer (blocker or activator)

FIG.1

Receptor Assay (2)

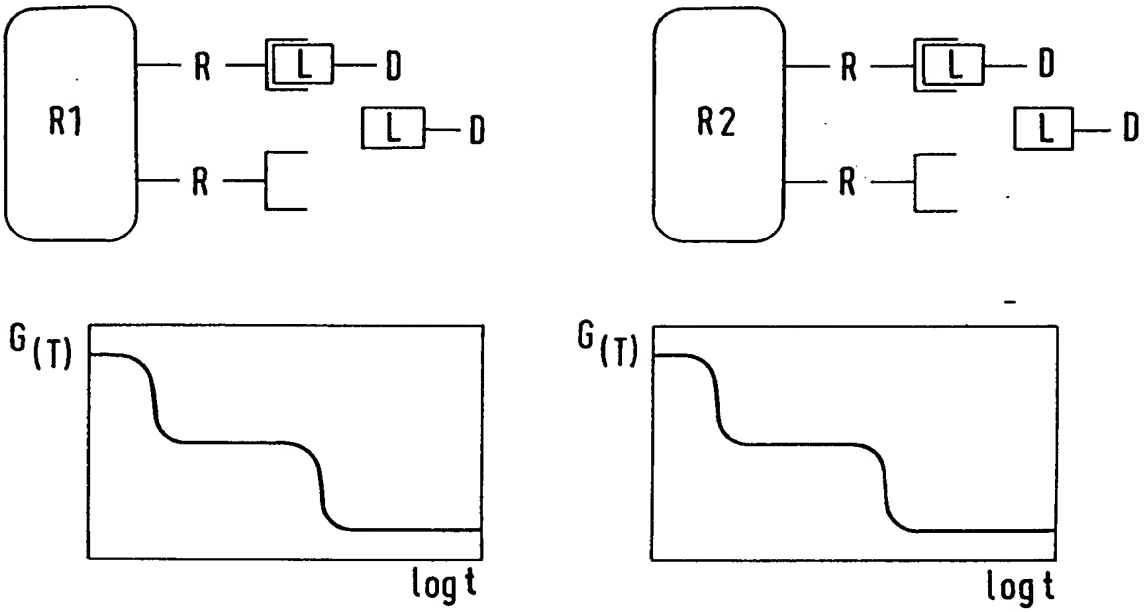


FIG.2

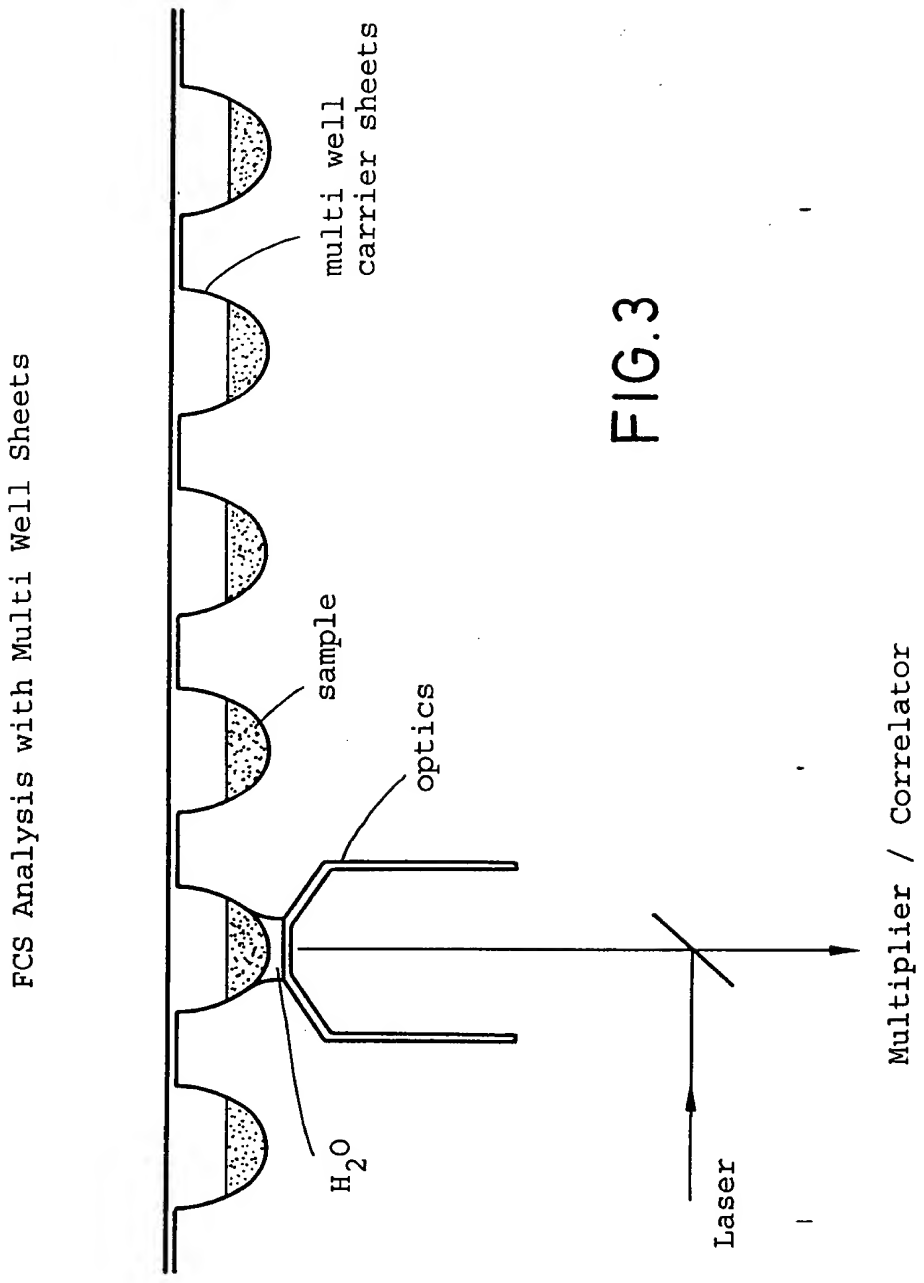

FCS Analysis with Multi Well Sheets
multi well carrier sheets
sample
optics
H_2O
Laser
Multiplier / Correlator

FIG.3

FCS - Determination of the Fitness of Mutants

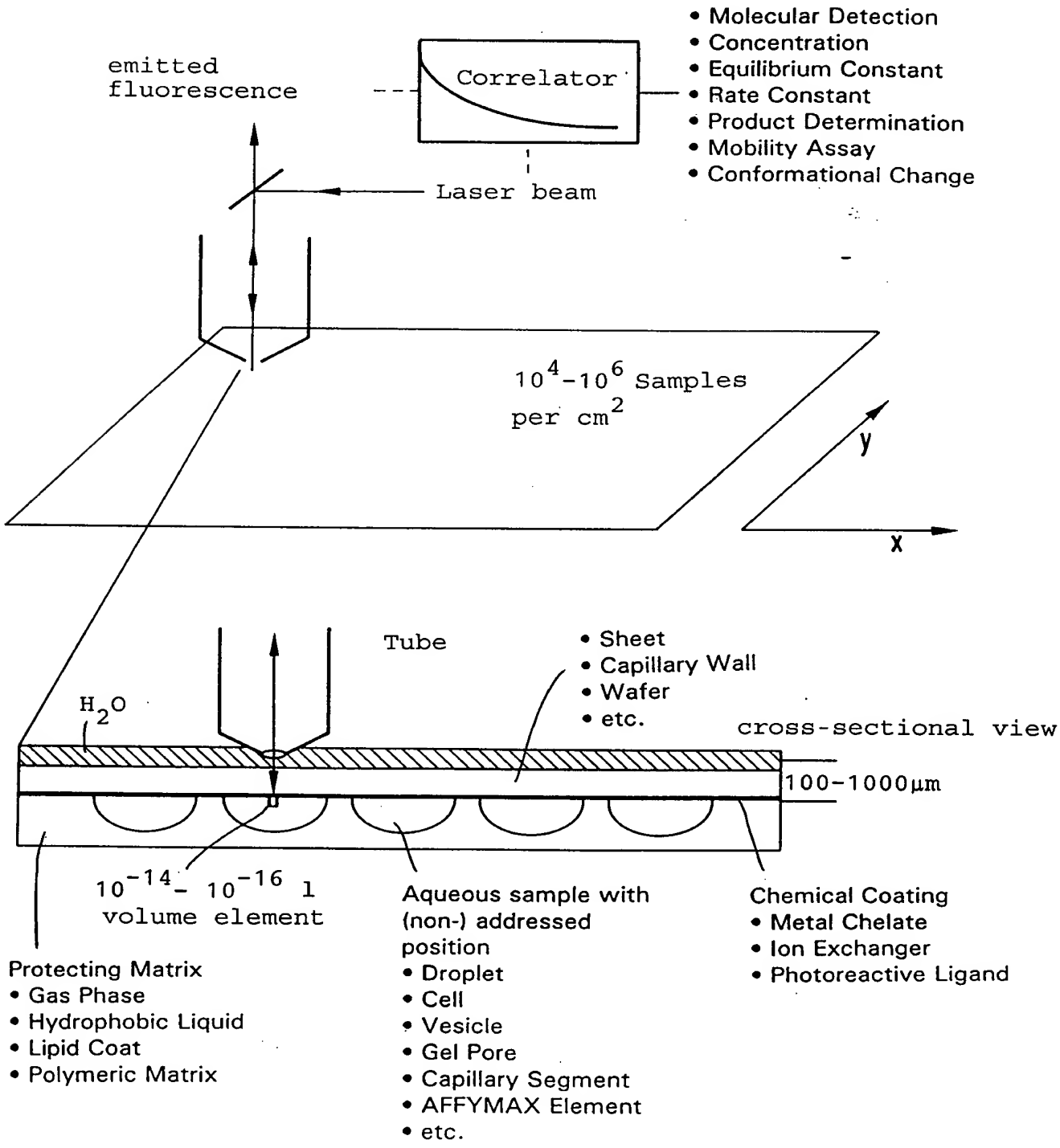


FIG. 4

Detection of Molecules on stationary structures through relative temporal change of the positional coordinates of the measuring volume

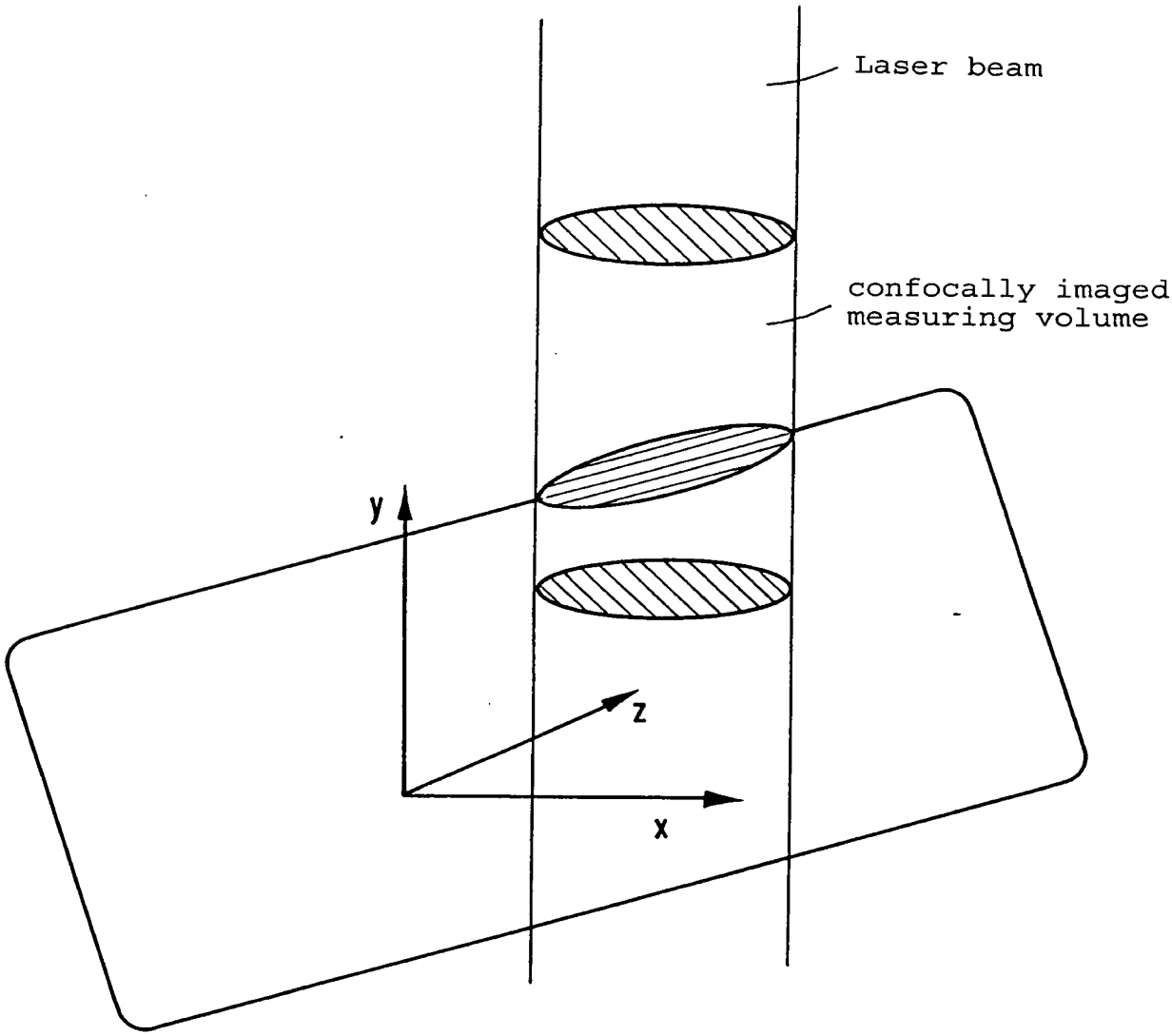


FIG.5

Detection of Single Molecules
in the Electric Trap

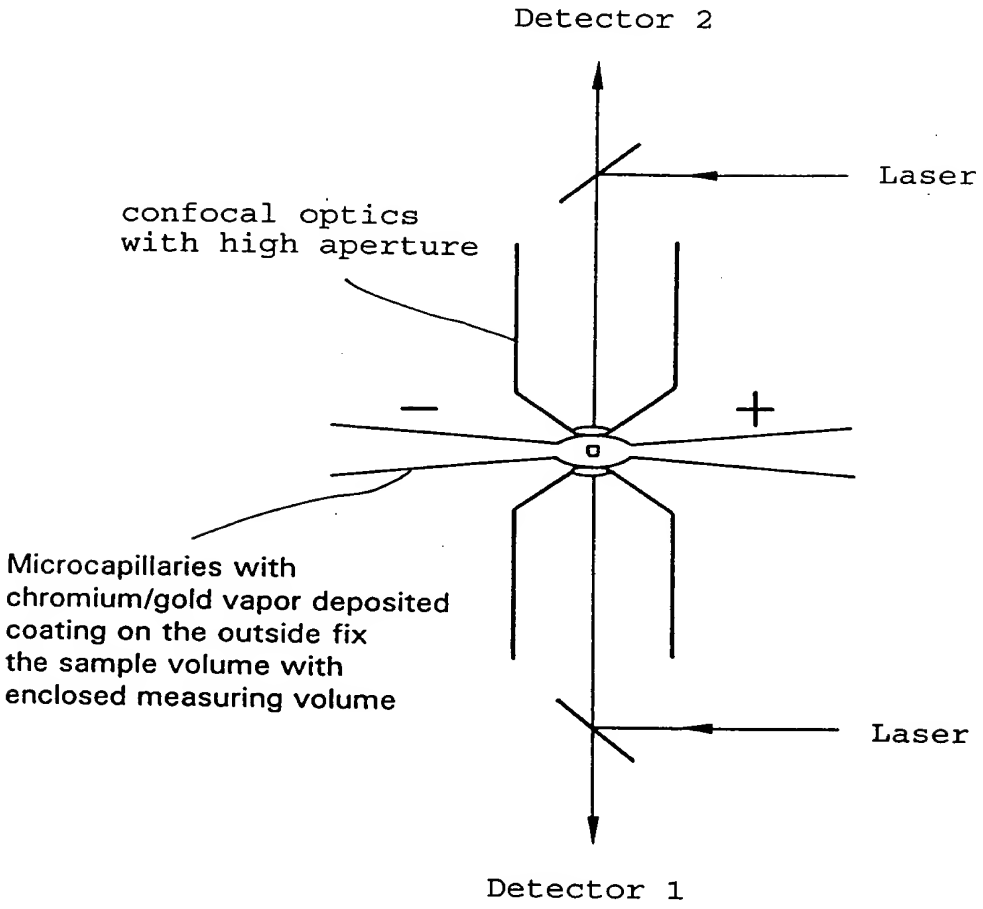
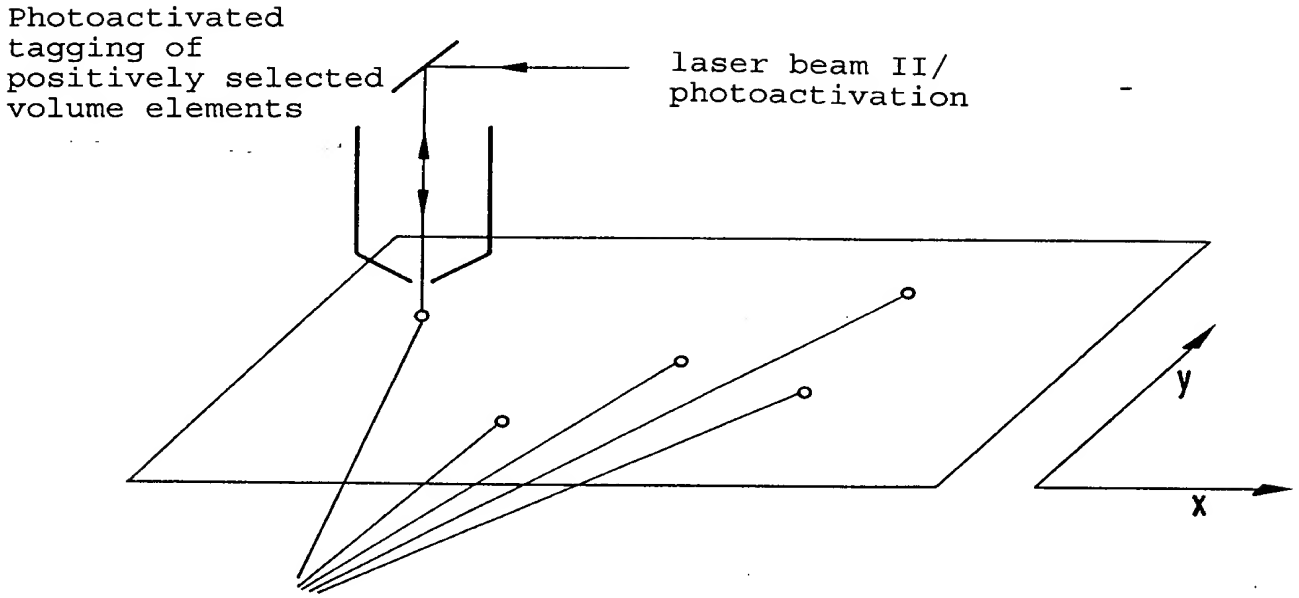


FIG.6

FCS - Tagging of the
Selected Genotypes



a) Physical access to optically
tagged volume elements

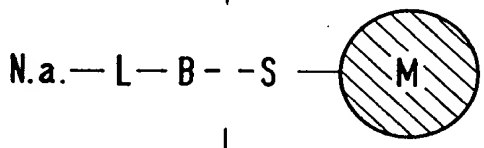
b) Light induced linking of the nucleic acid
of selected volume elements to affinity ligands
- at the carrier surface
- to soluble ligands

FIG.7

Preparation of the DNA/RNA of FCS Selected Genotypes

Mixture of all nucleic acids after phenotype evaluation:

Plasmid DNA Cellular DNA rRNA/tRNA/mRNA	Excess from untagged volume elements
Plasmid DNA Cellular DNA rRNA/tRNA/mRNA (containing sequences encoding selected phenotype)	Minor amounts from untagged volume elements in which affinity ligands (L) have been photochemically coupled (laser induced) to the nucleic acids present in the respective volume element



cDNA synthesis or enzymatic amplification

N.a.; Nucleic acid.
L; Ligand with specific nucleic acid affinity which can be photochemically coupled covalently and preferably reversibly to a nucleic acid (e.g. a psoralen derivative). The ligand is preferably linked to a substituent which allows for subsequent enrichment of the nucleic acids. For instance, this can be a hydrophobic substituent to purify nucleic acids by reversed phase chromatography. For affinity chromatography, substituents such as biotin (B) are the obvious suitable ones so that the nucleic acids can be enriched through (strept)avidin complexing (S) with appropriately modified magnetobeads (M) or surfaces.

FIG.8

FLUCS Analysis of Complex Mixtures of Substances after Chromatographic Separation in Fractions

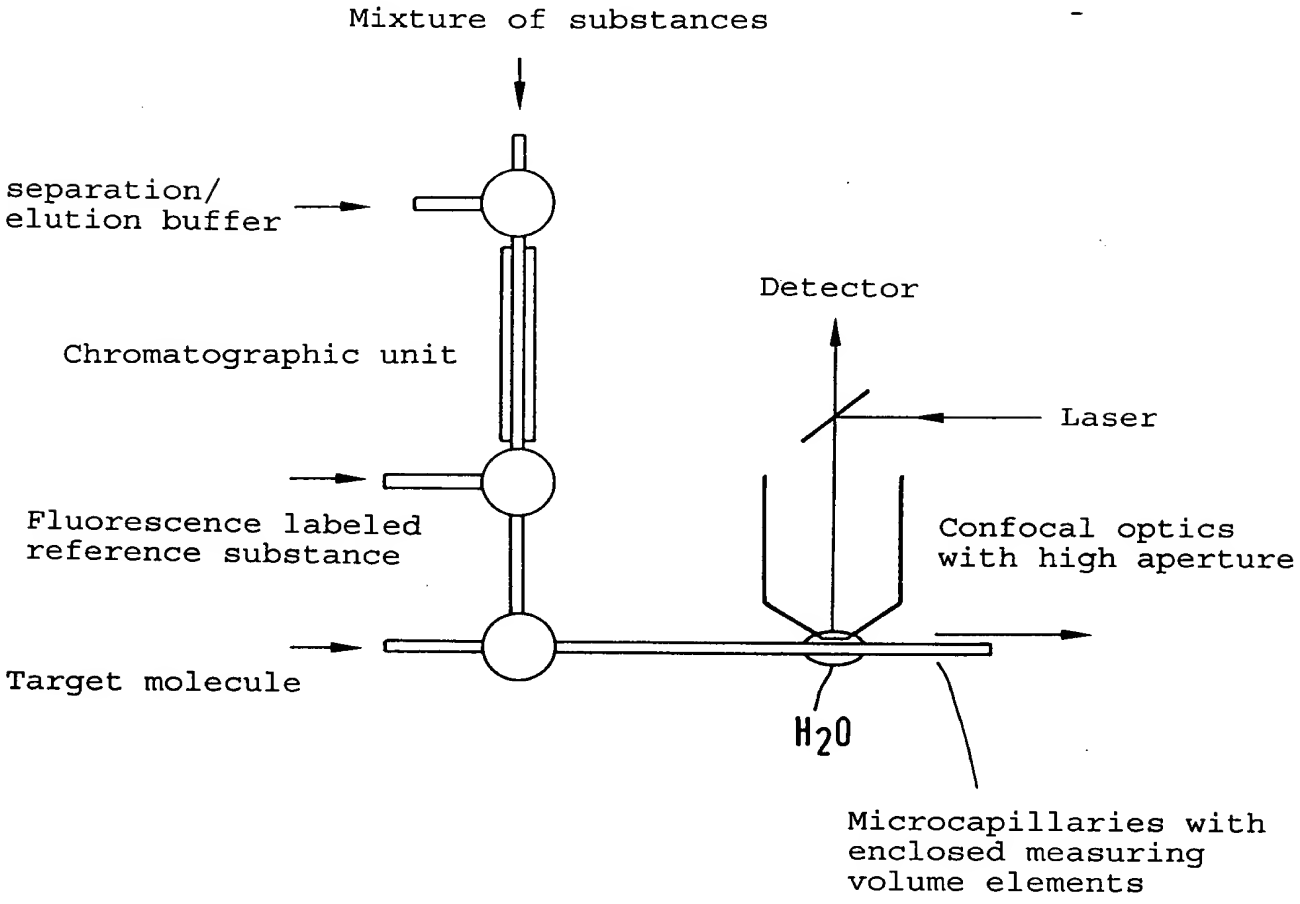


FIG.9

Laser Correlation Microscope

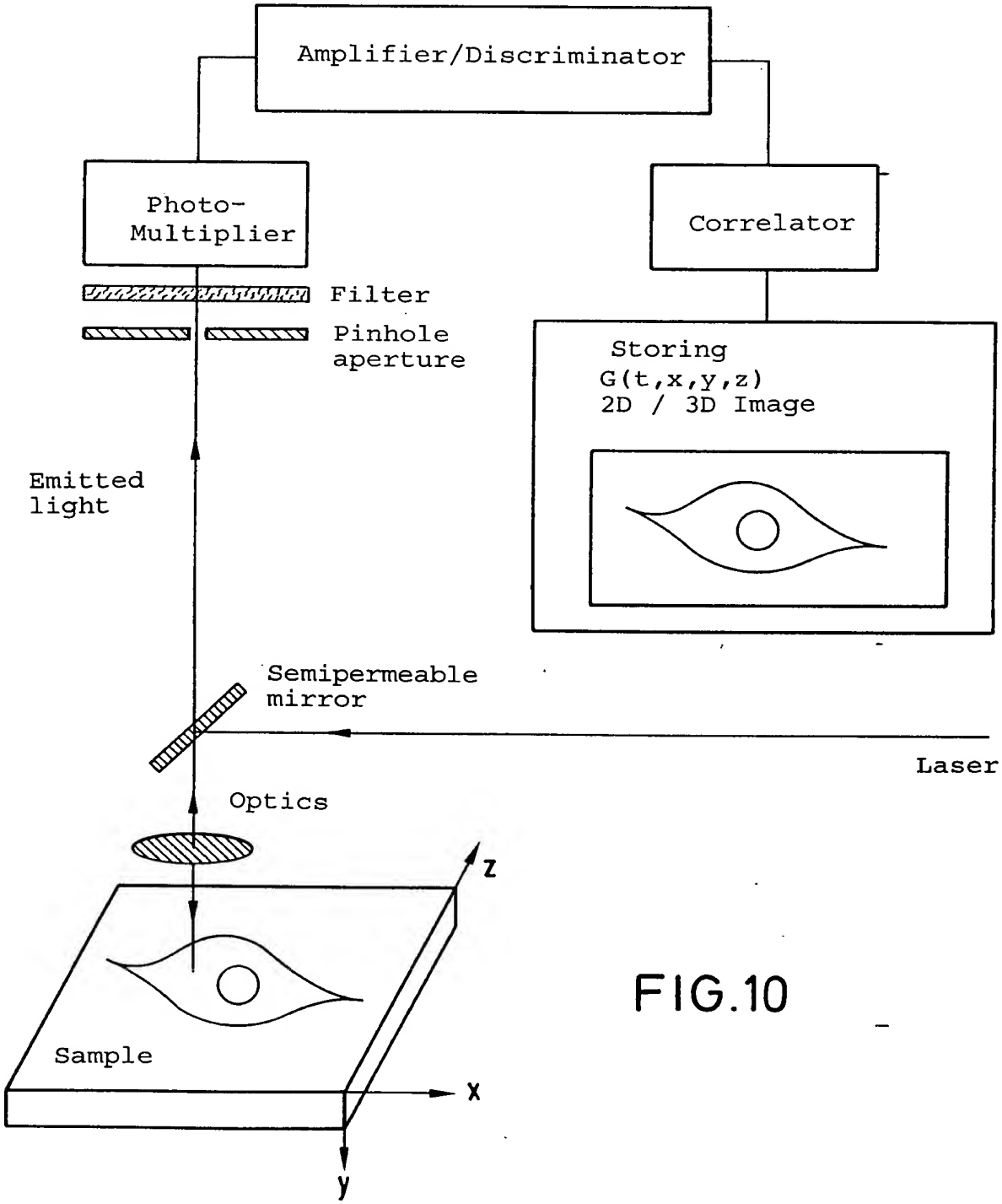


FIG.10

Selection of Possible Assays

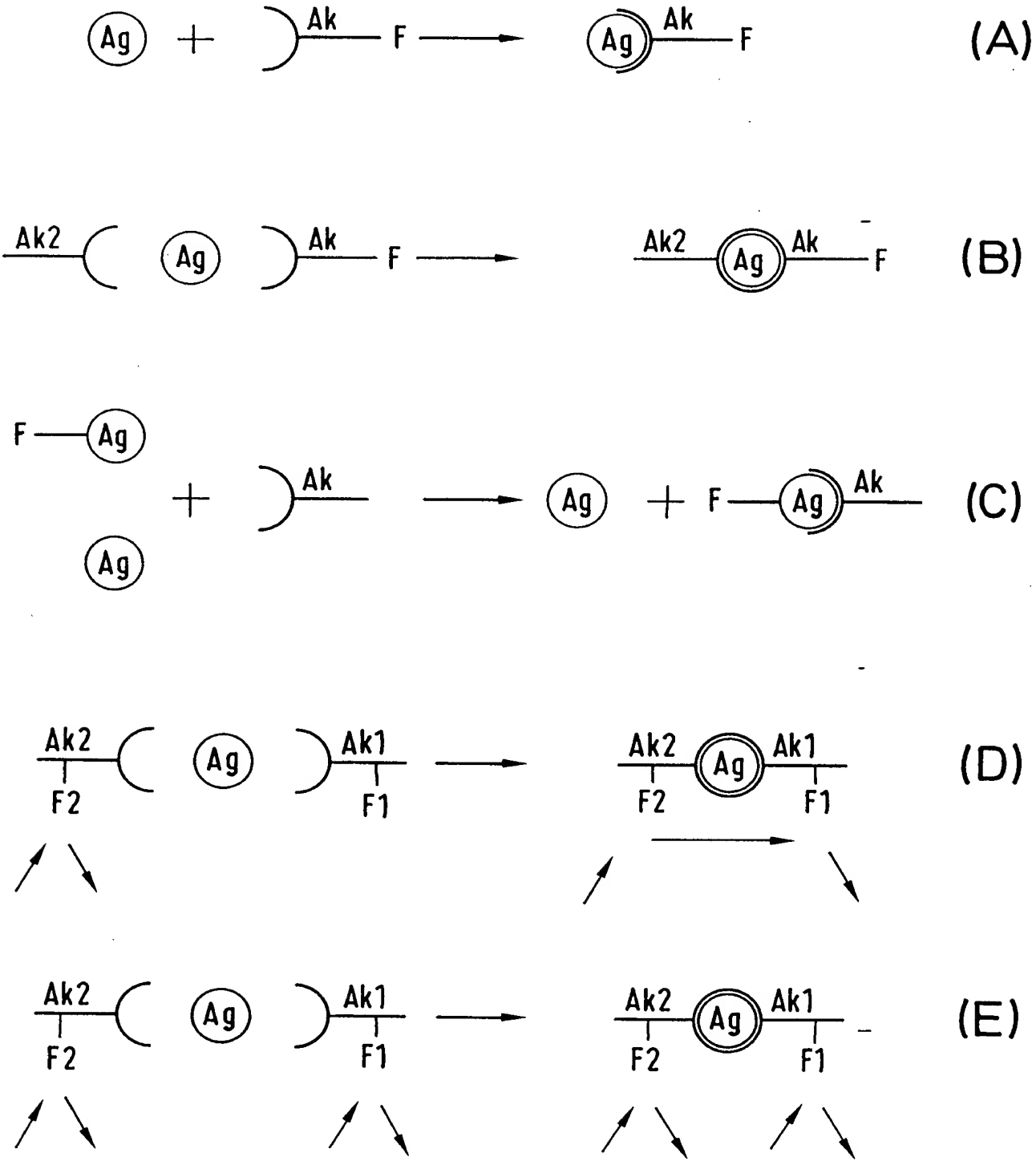


FIG.11

Electrophoresis Cell

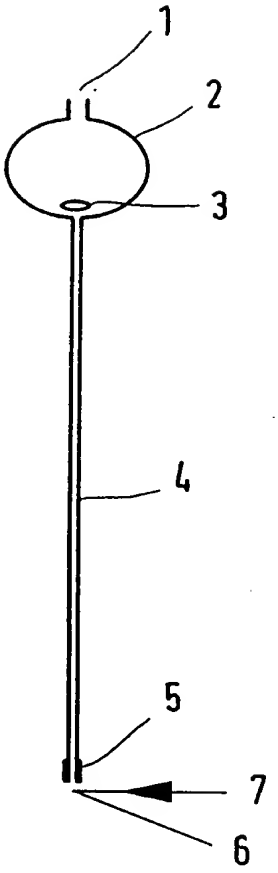


FIG.12

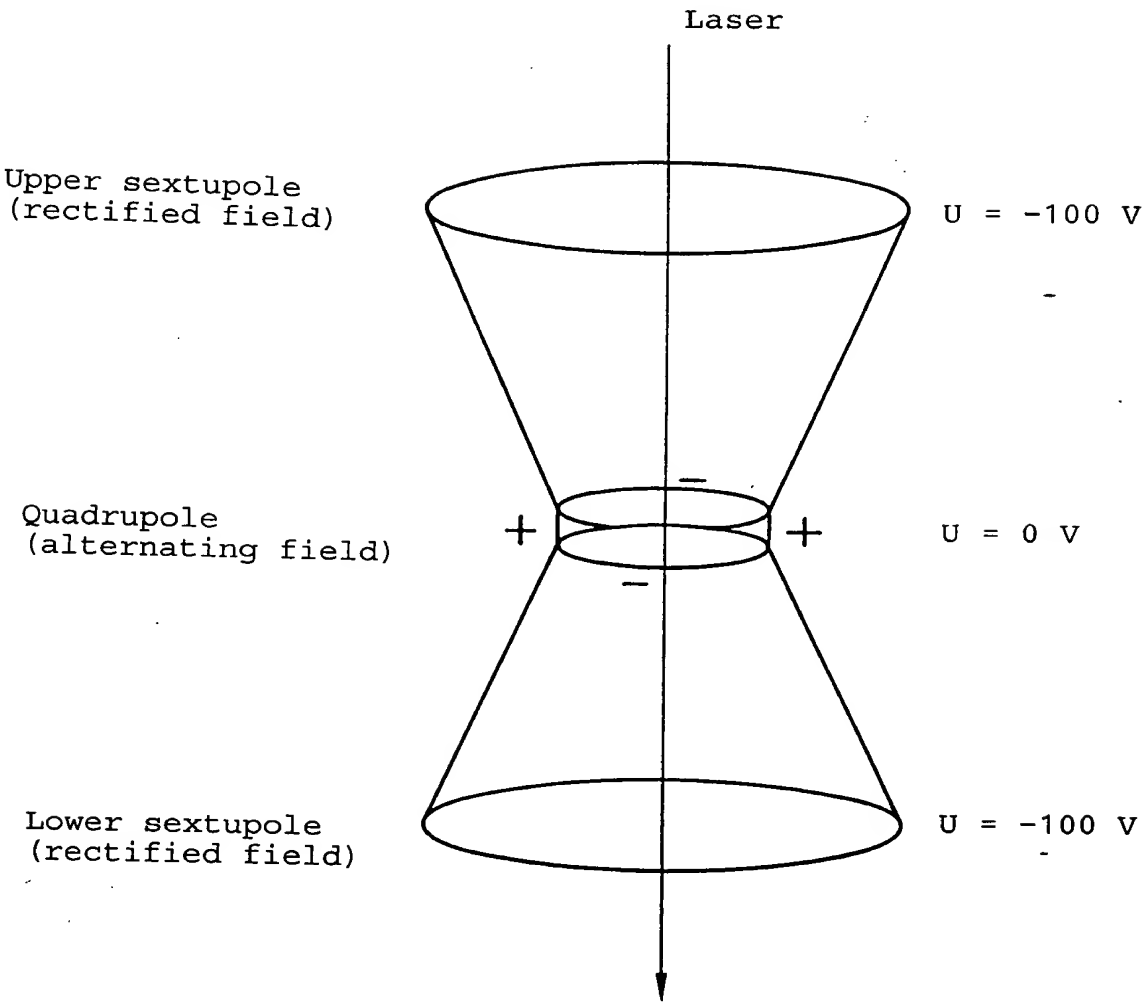

Laser
Upper sextupole
(rectified field)
U = -100 V
Quadrupole
(alternating field)
U = 0 V
Lower sextupole
(rectified field)
U = -100 V

FIG.13

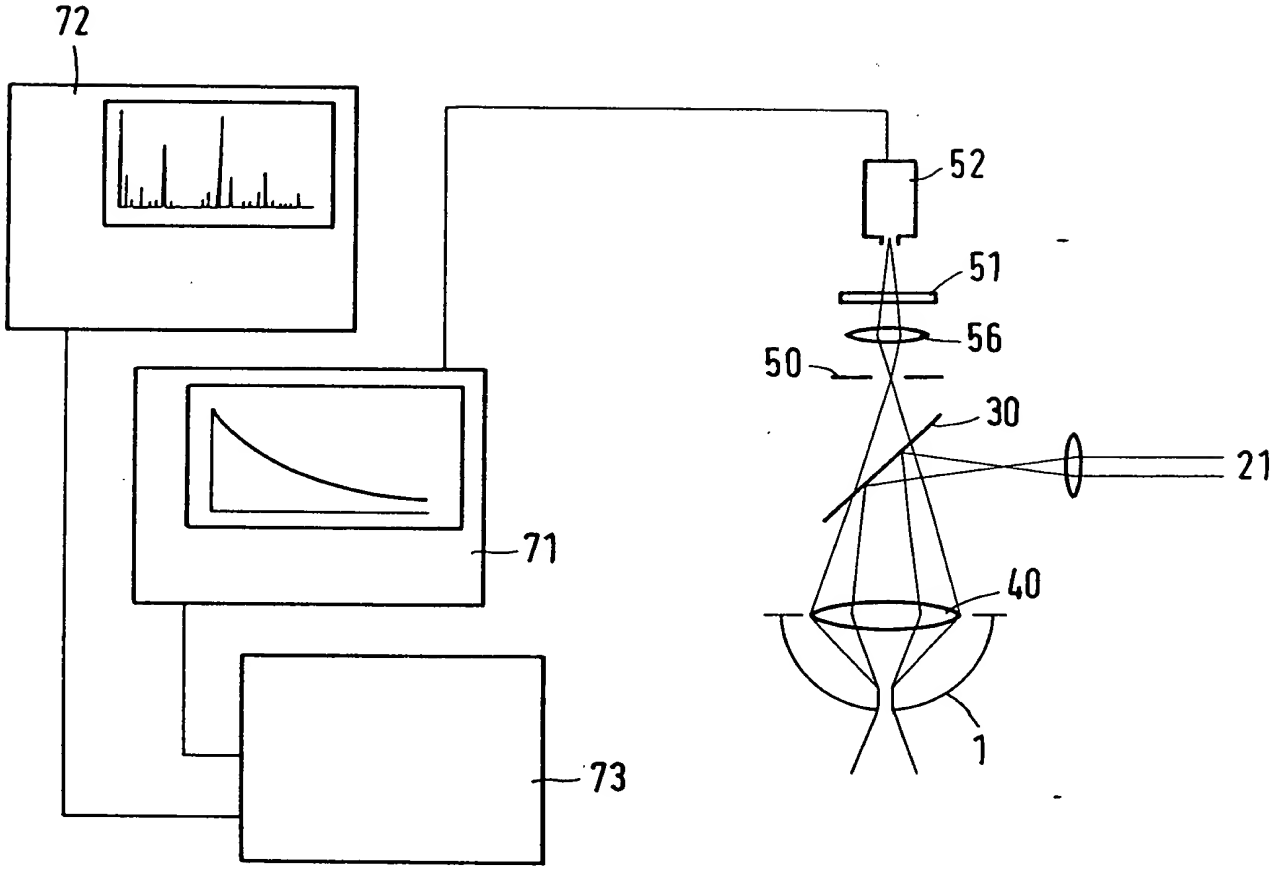


FIG. 14

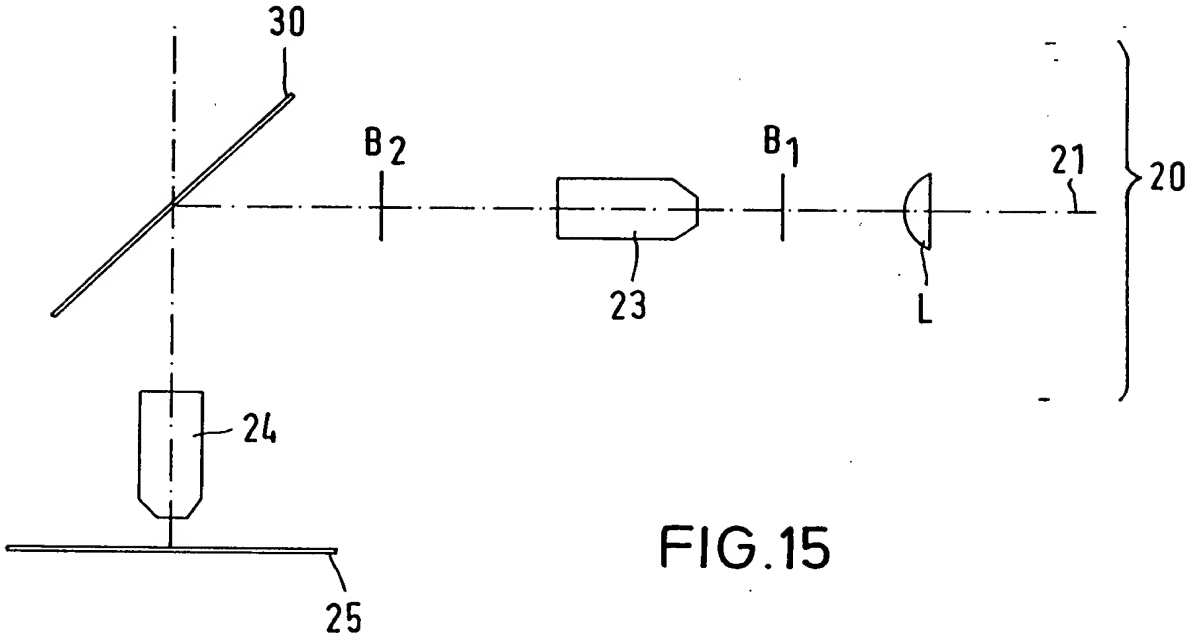


FIG.15

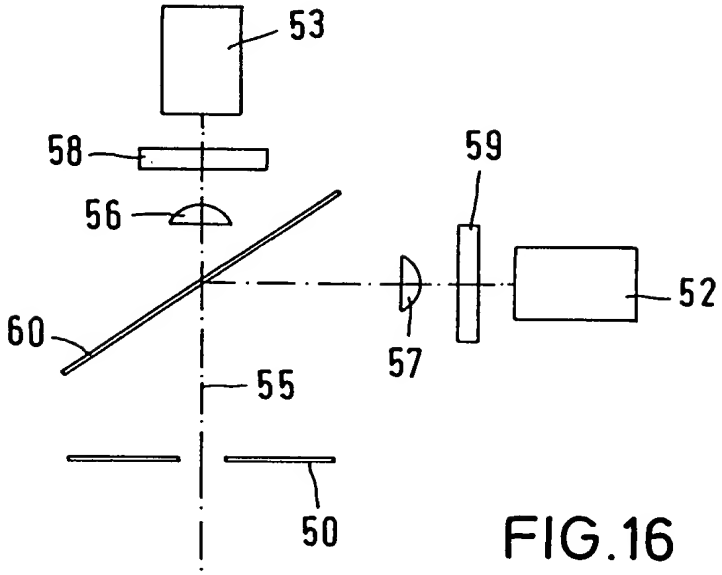


FIG.16

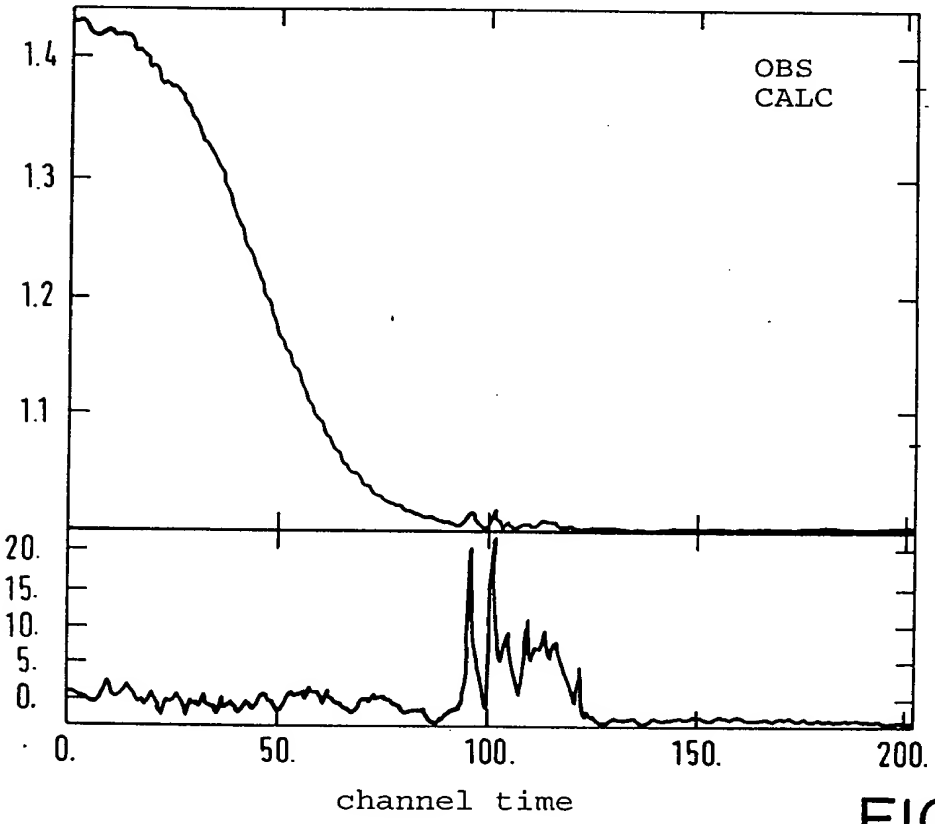


FIG.17a

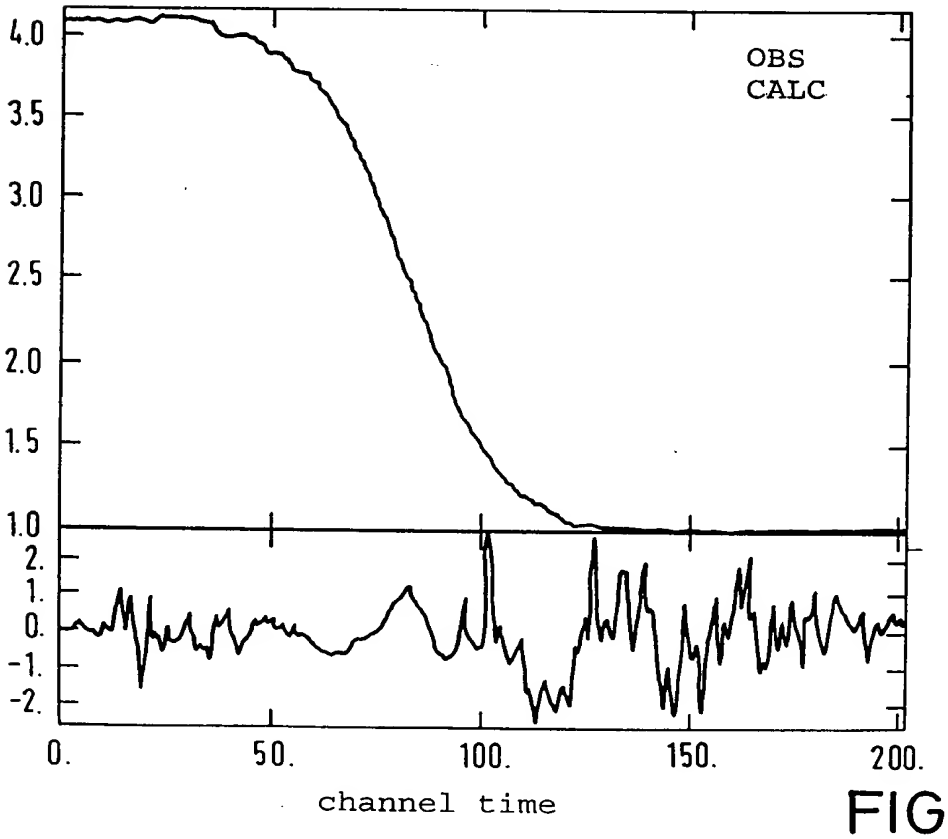


FIG.17b

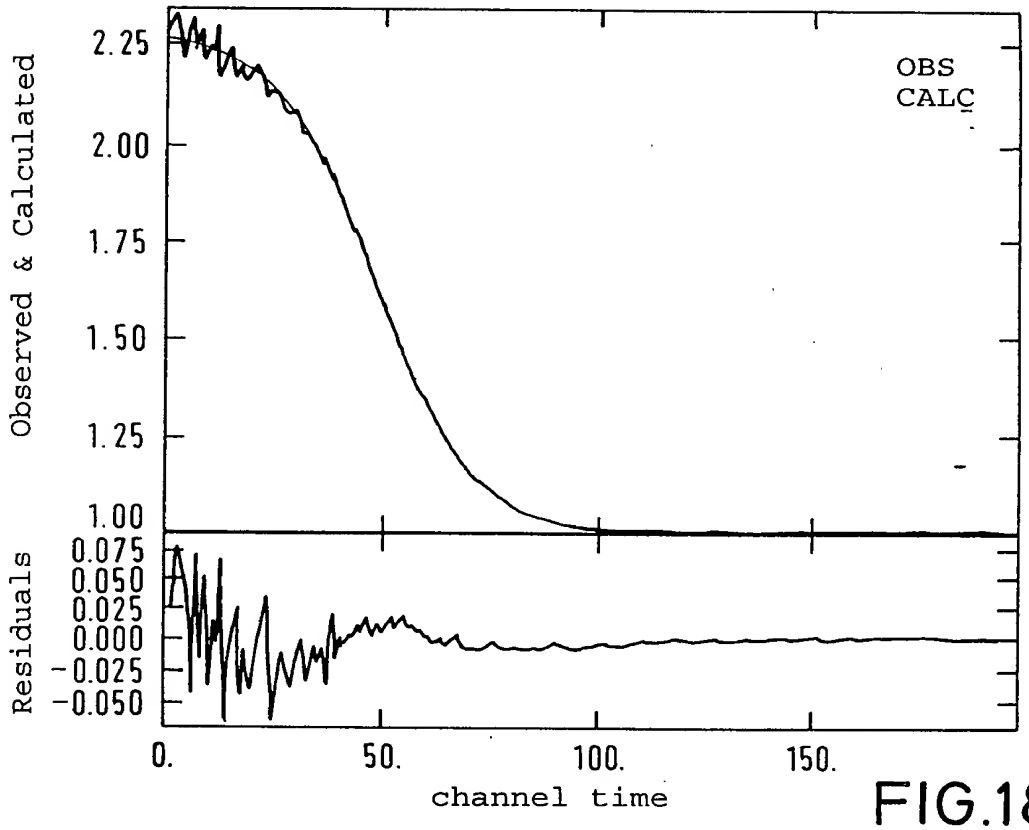


FIG.18a

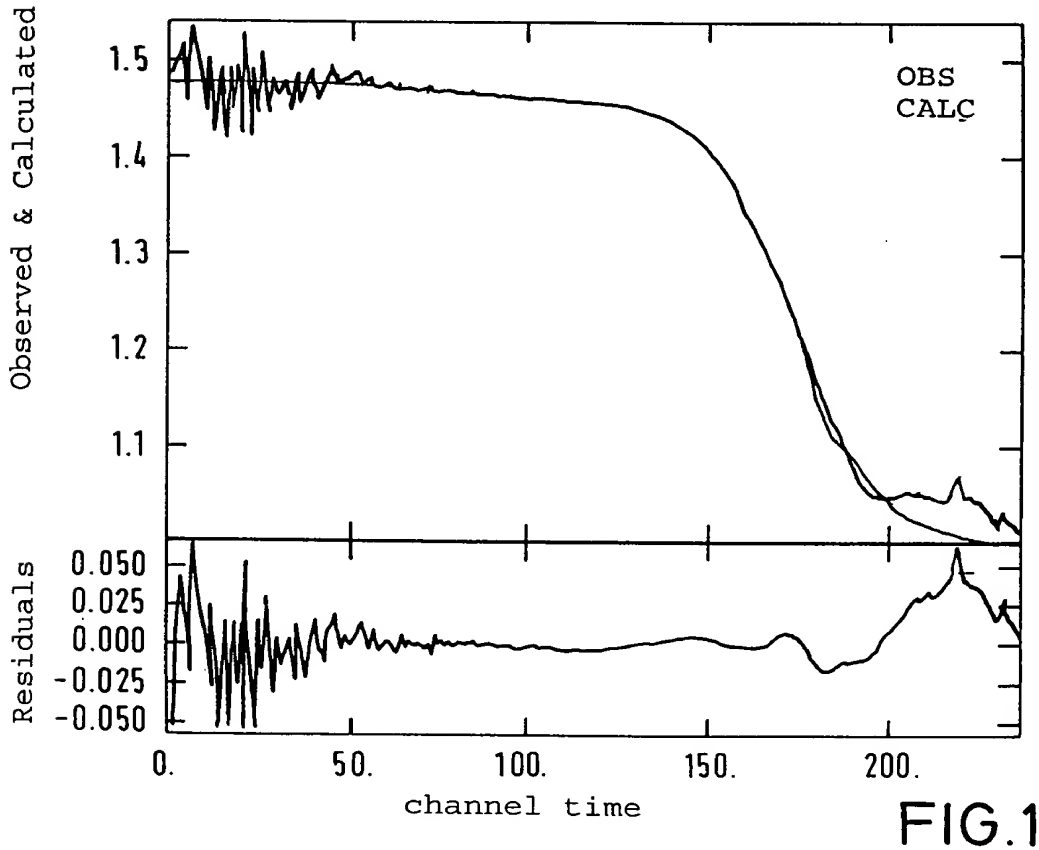


FIG.18b

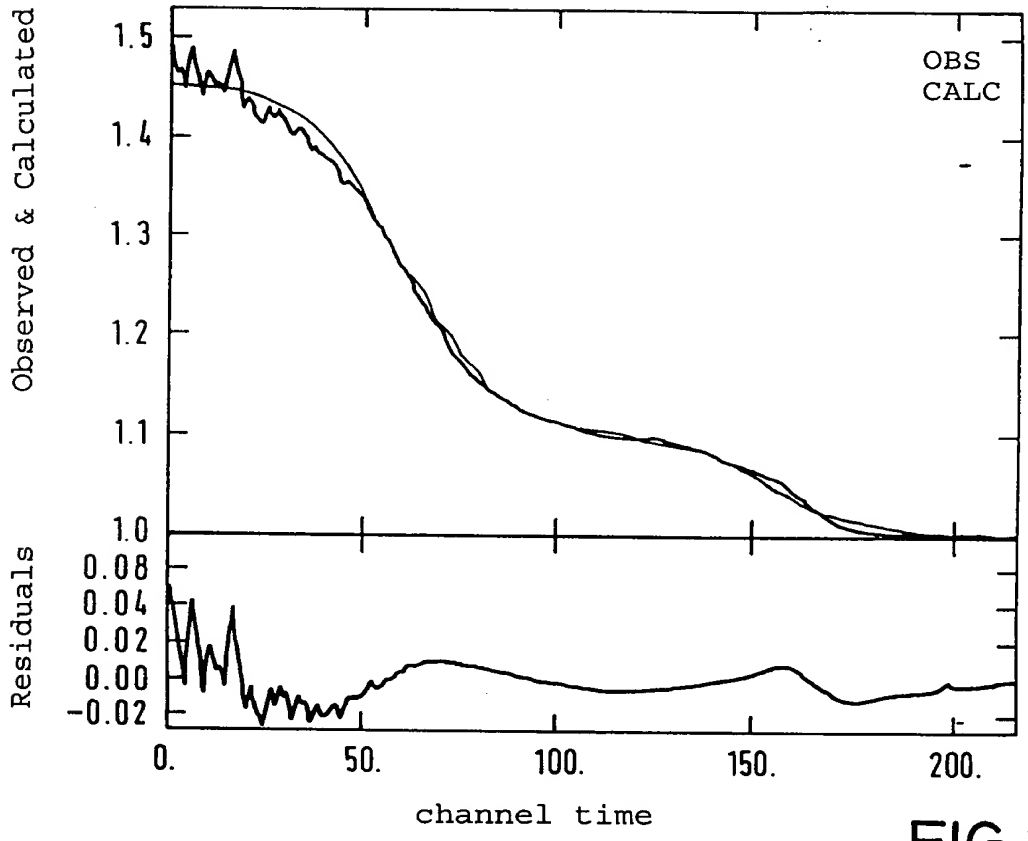

Observed & Calculated
1.5
1.4
1.3
1.2
1.1
1.0
OBS
CALC
Residuals
0.08
0.04
0.02
0.00
-0.02
0.
50.
100.
150.
200.
channel time

FIG.18c

Determination by FCS of the Dissociation Behavior of Complexes in Experiments Performed in Parallel

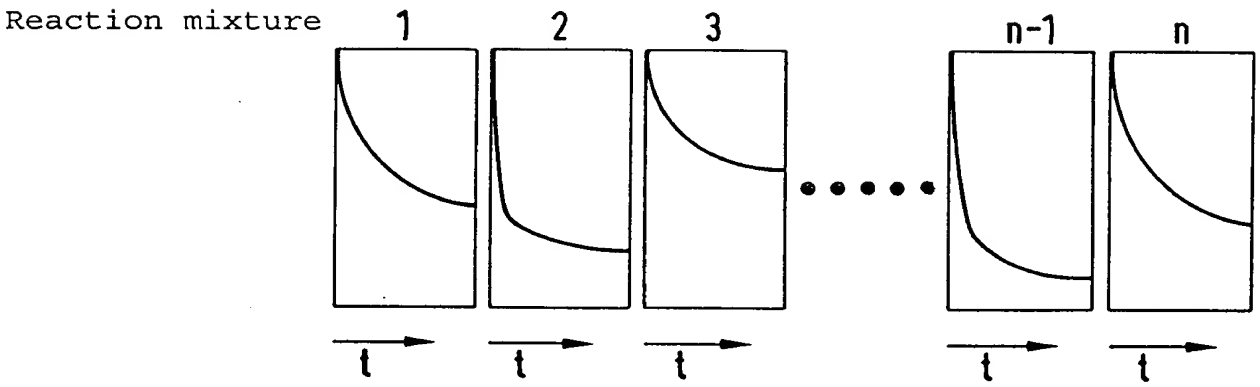
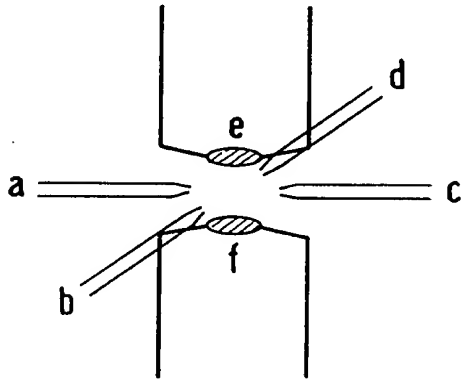


FIG.19

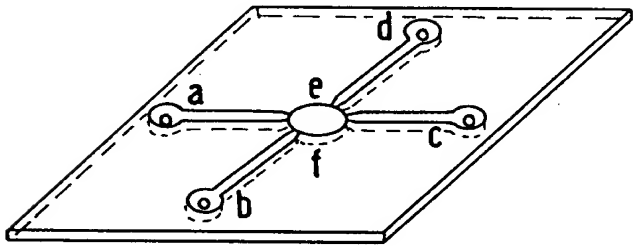
Different Embodiments of the Electric Trap According to the Invention

FIG.20a



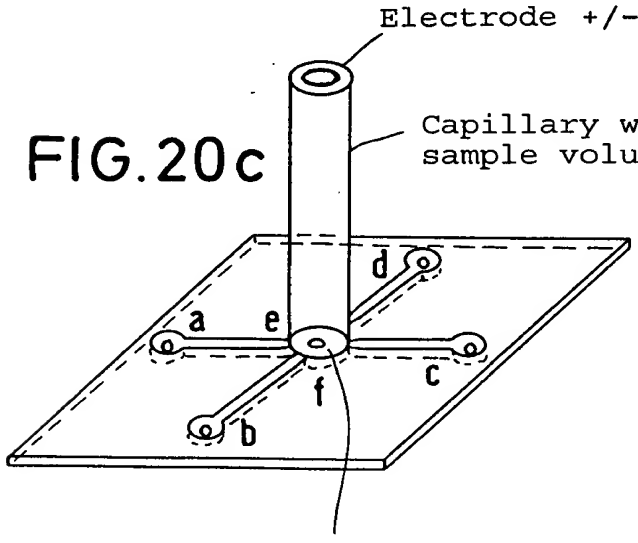
a, b, c, d as quadrupole electrodes (metal coated Neher tips or metal vapor coated electrodes on microstructures on flat sample carriers (silicone, glass, and other basis materials)).
e,f as sextupole electrodes (e.g. as metal vapor coated emergence lens of one or two objectives. Adjustment is performed by x,y,z adjustment.

FIG.20b



Use of flat carriers with etched electrode channels (or forms made by LIGA technique) through which charged molecules can be controlled with respect to their migration in the electric field, can be led in or out. The bottom plates at e and f can be objectives coated as sextupole electrodes or metal vapor coated coverings.

FIG.20c

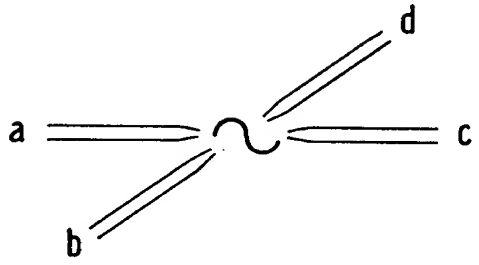


Use of (b) in combination with a sample dispenser system consisting of a capillary made of mineral materials (e.g. glass, silicon, etc.) or plastics (e.g. Teflon) for the reception of large sample volumes having an electrode at the end of the capillary (about +/- 0-100 Volt) and a collecting electrode at earth potential (0 Volt).

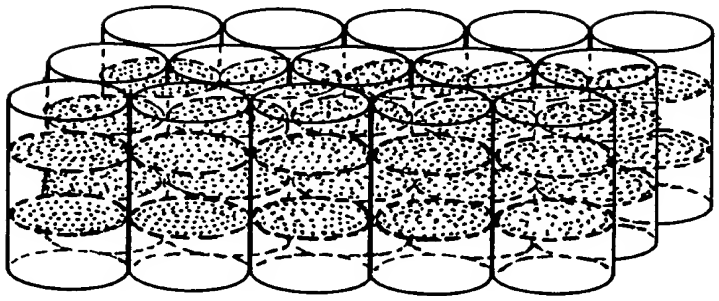
Collecting electrode with earthing (potential 0 V) and Pinhole for ions to pass into the quadrupolar field

Molecular Detection

FIG.21a



If target molecules are present within the quadrupole or sextupole field the molecules can be set into forced motion by a random alternating field over the electrodes a, b, c, d. They thus become countable according to the invention.



The position of a molecule within the trap is recognized by a multielement detector. By active feedback the quadrupole/sextupole field is adjusted such that the molecule gets fixed in its position within a defined area/volume element.

FIG.21b

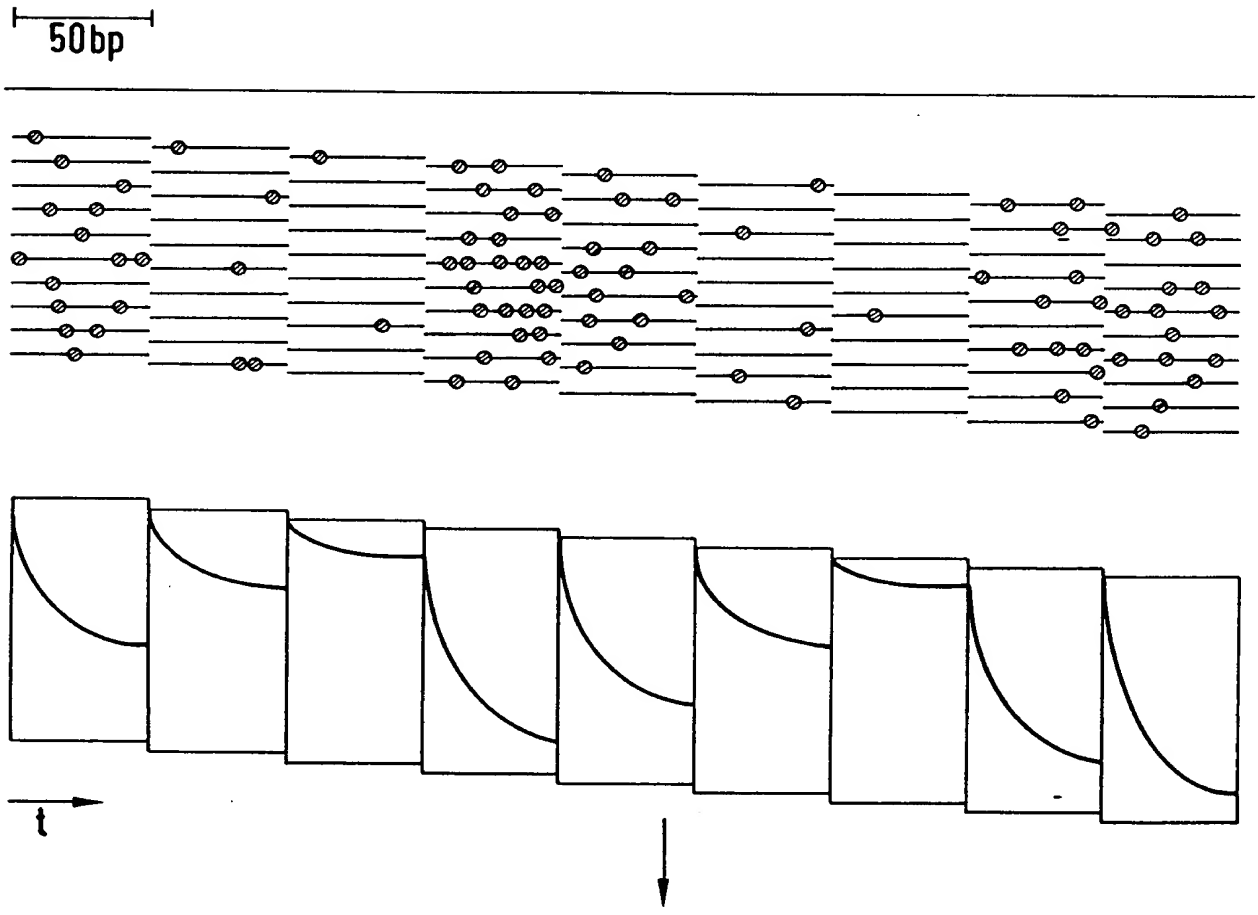


FIG.22

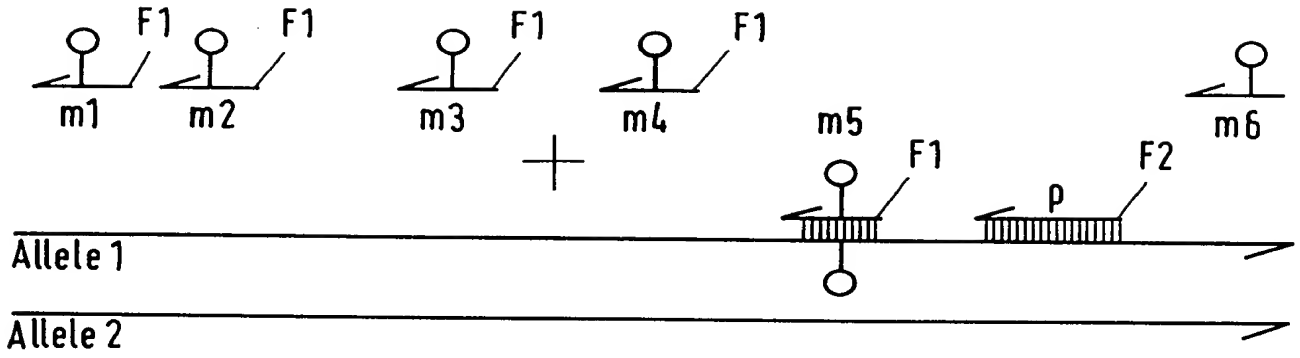


FIG.23

Small Excitation Volumes (a) and
Small Measuring Volumes (b) and Small
Volumes with Parallel Measurements (c)

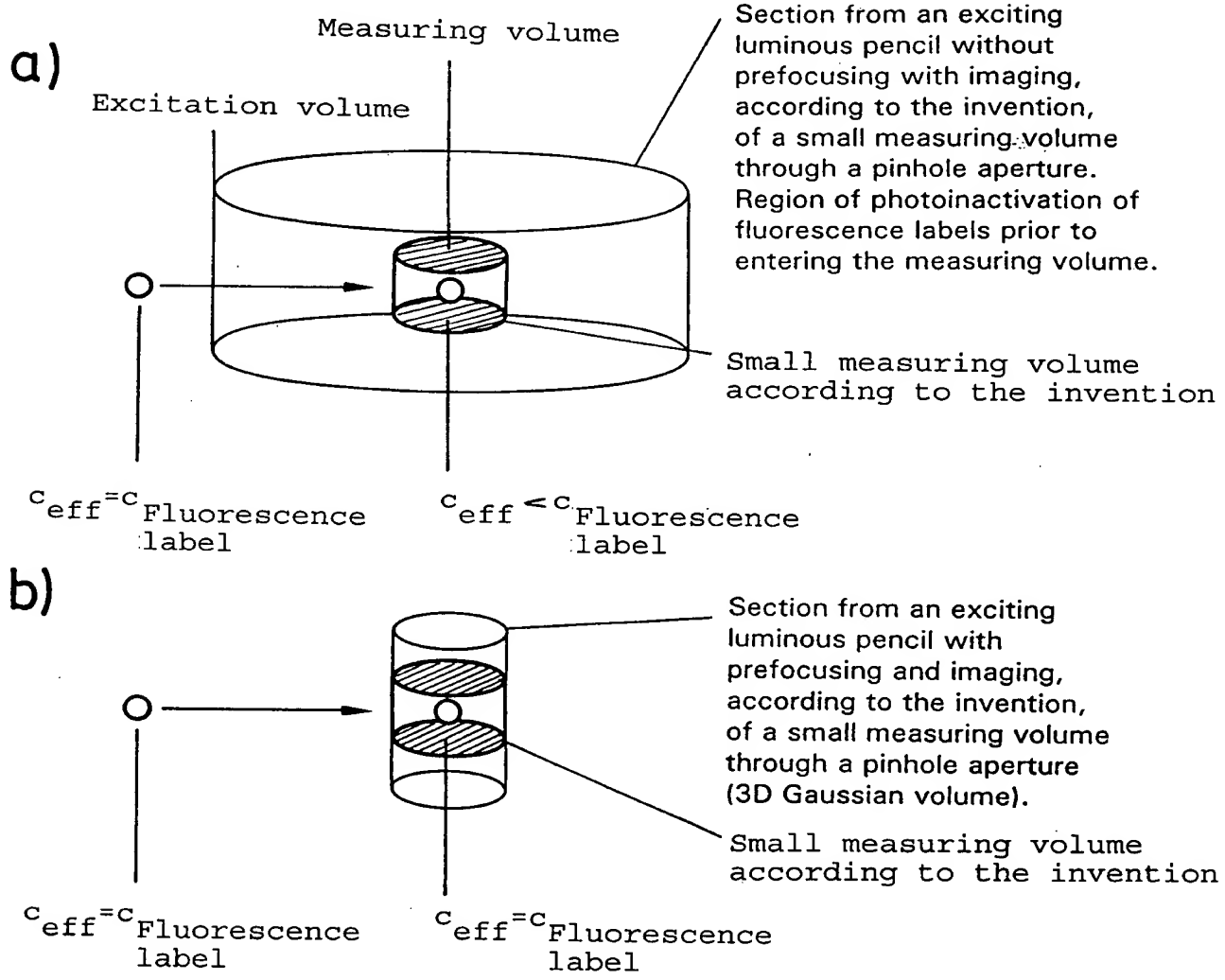
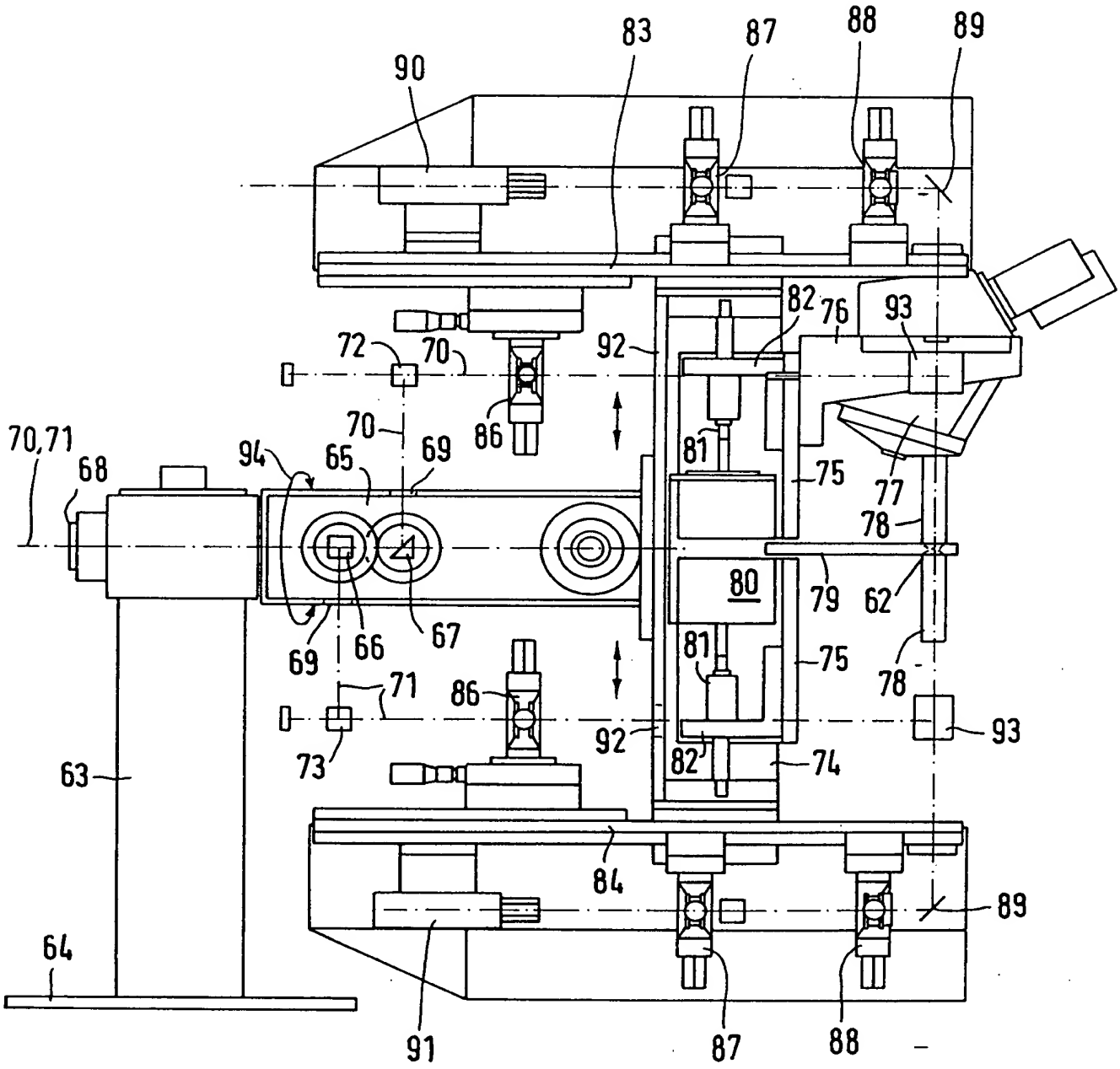


FIG.24

FIG.25


83
87
88
89
90
82
76
93
72
70
92
70
86
81
70,71
68
94
65
69
75
77
78
80
79
62
69
66
67
81
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78
71
86
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93
73
82
74
63
84
89
64
91
87
88

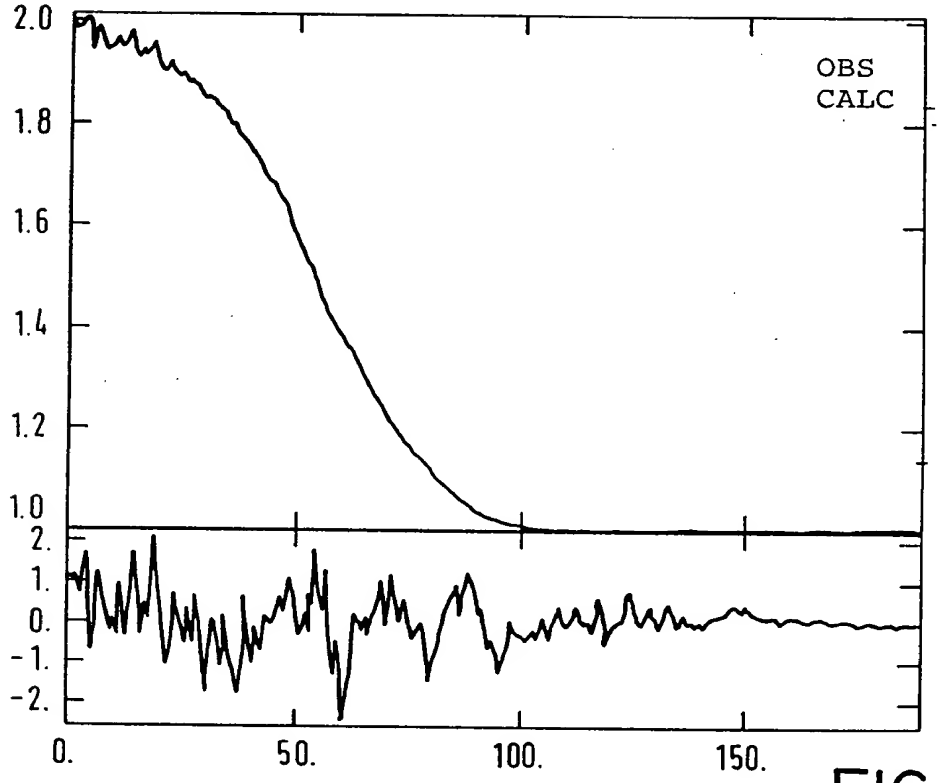


FIG.26a

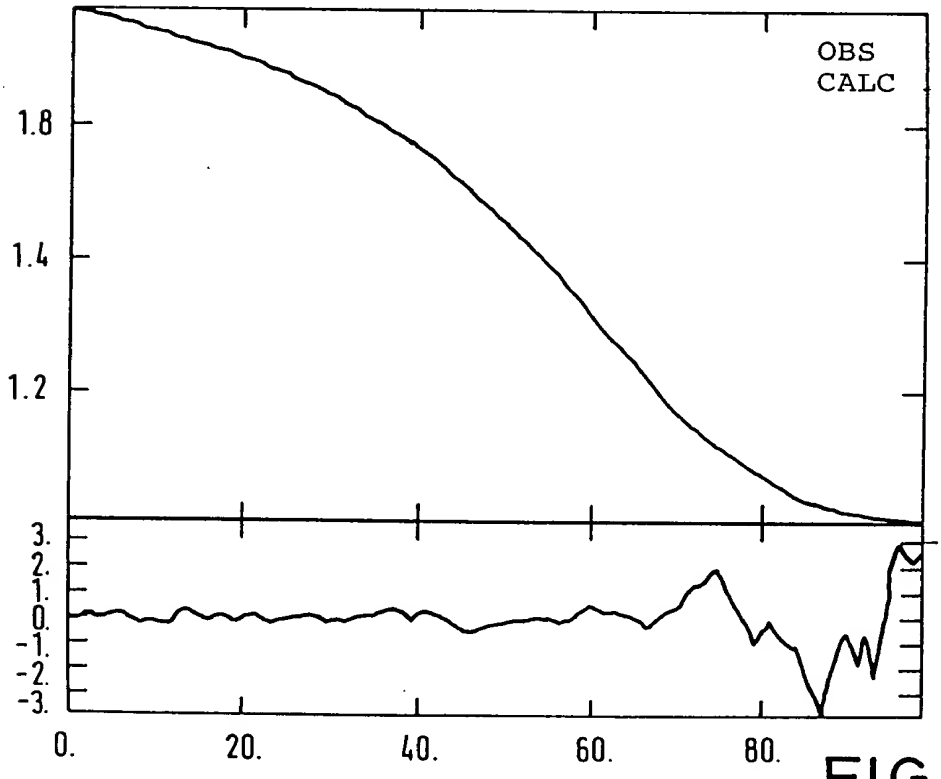


FIG.26b

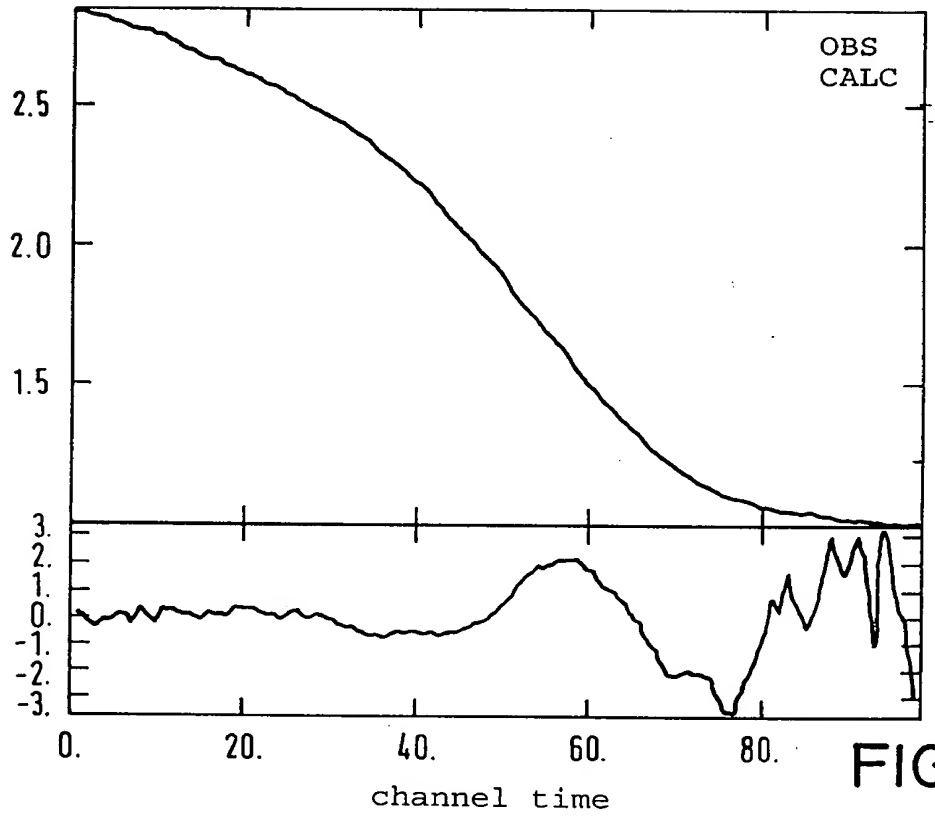


FIG.26c

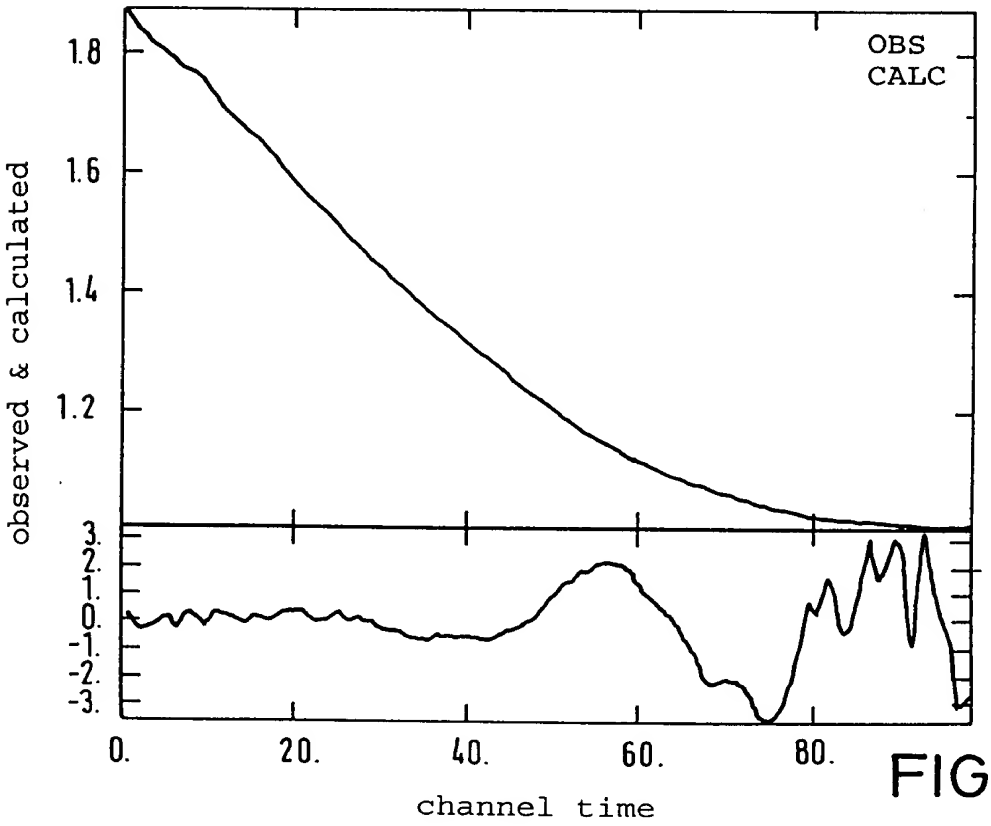


FIG.27

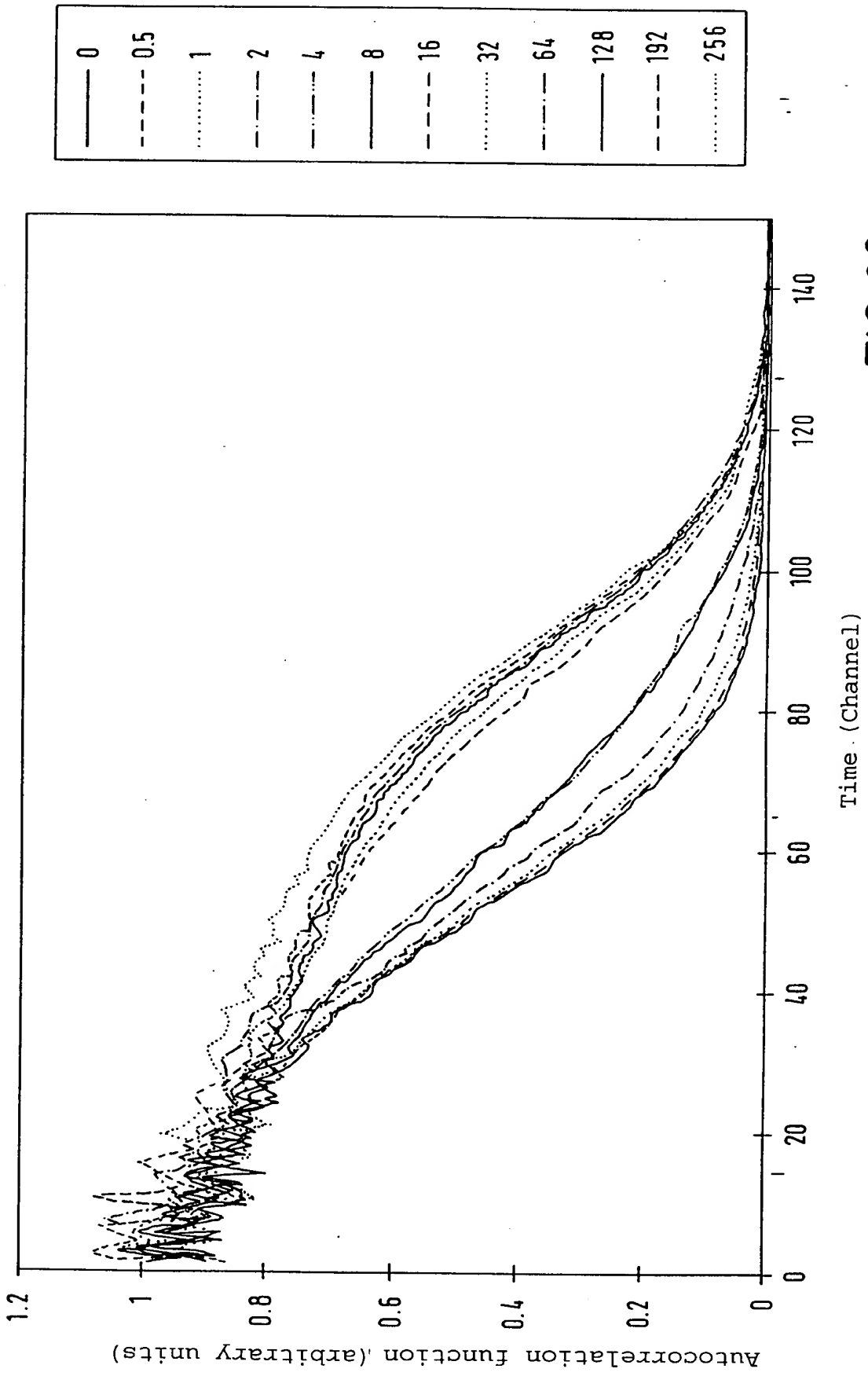

0
0.5
1
2
4
8
16
32
64
128
192
256
Autocorrelation function (arbitrary units)
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
140
Time (Channel)

FIG.28a

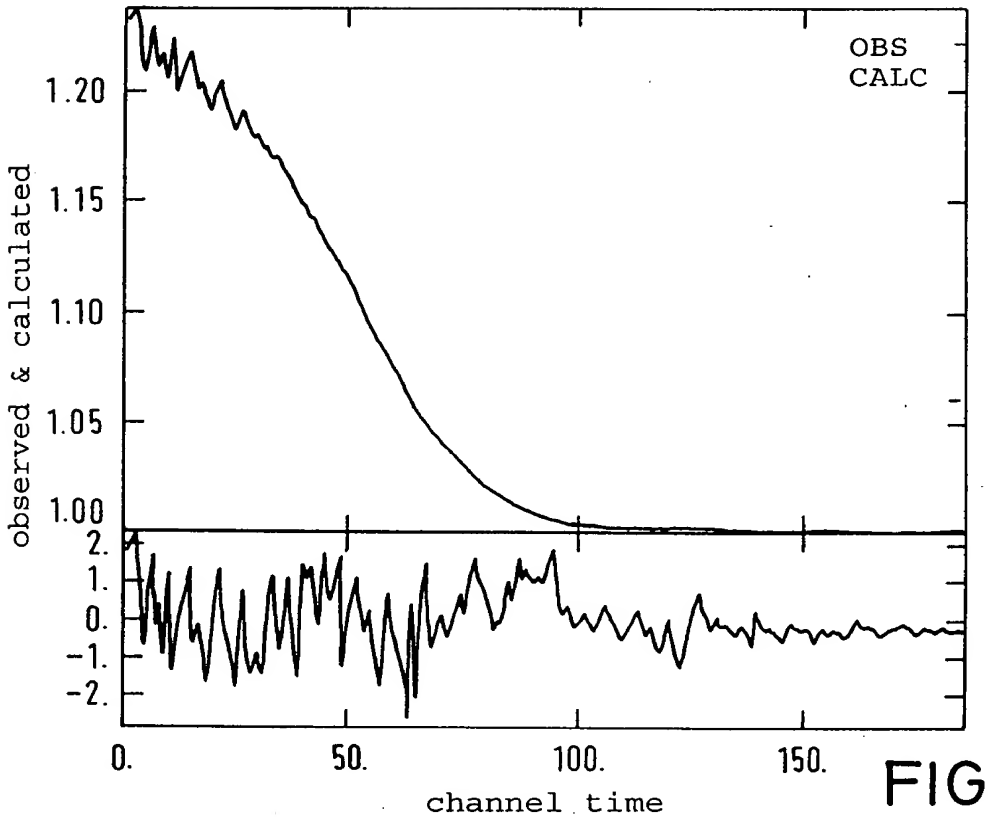


FIG.28b

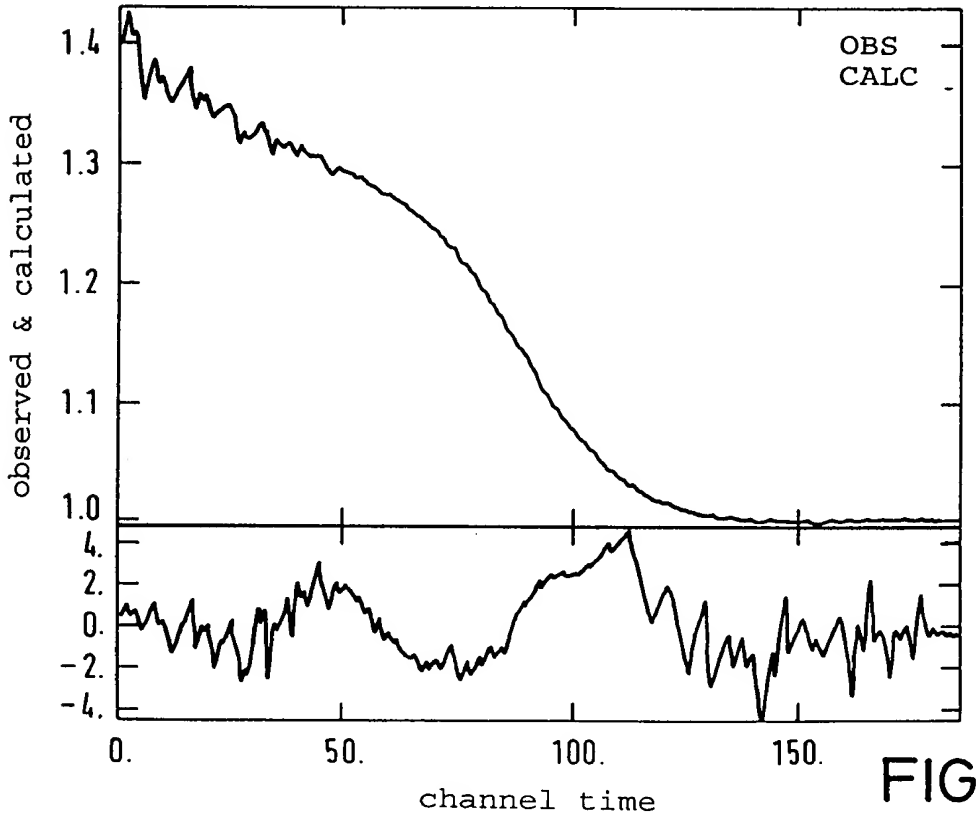


FIG.28c

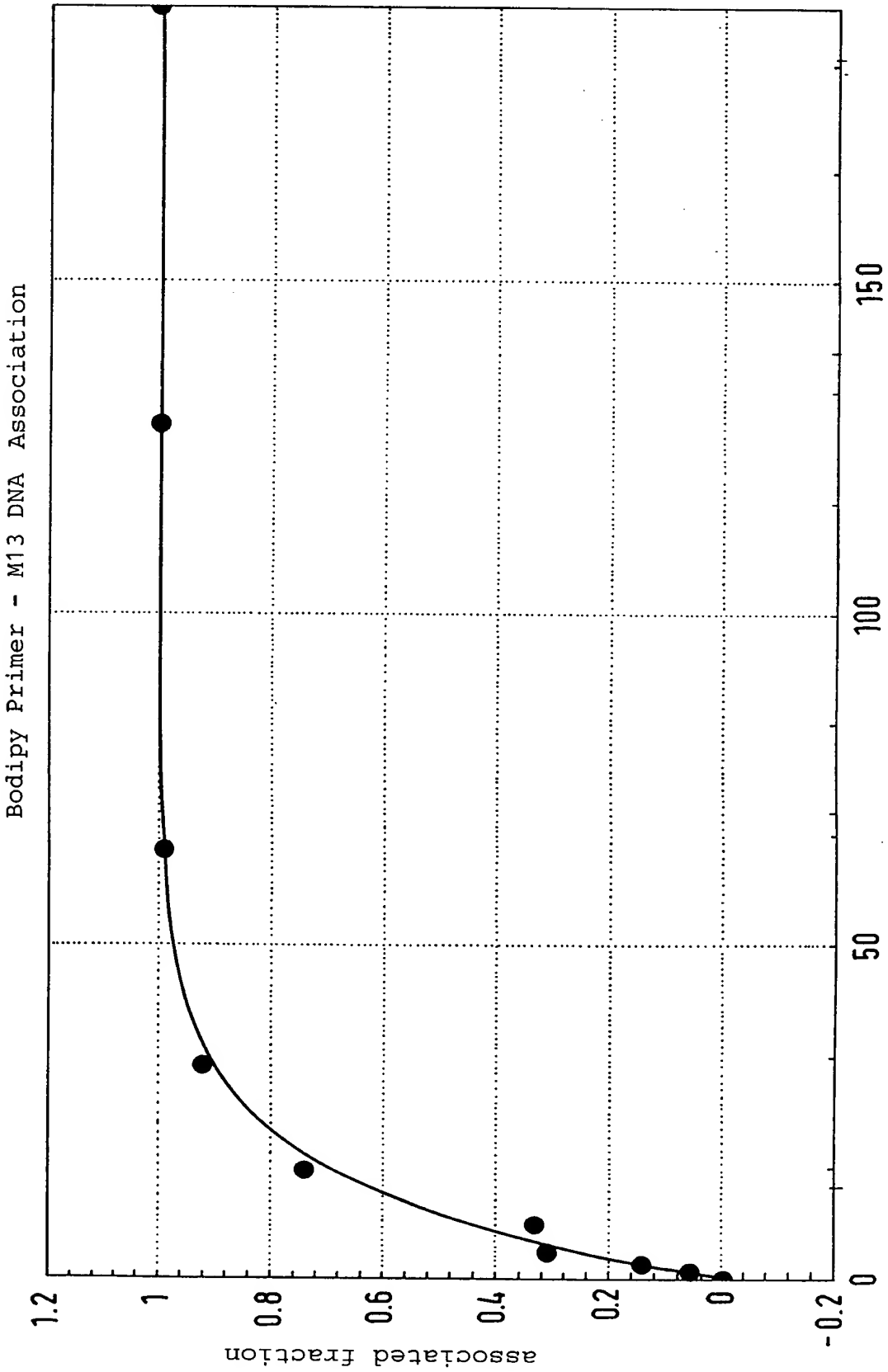
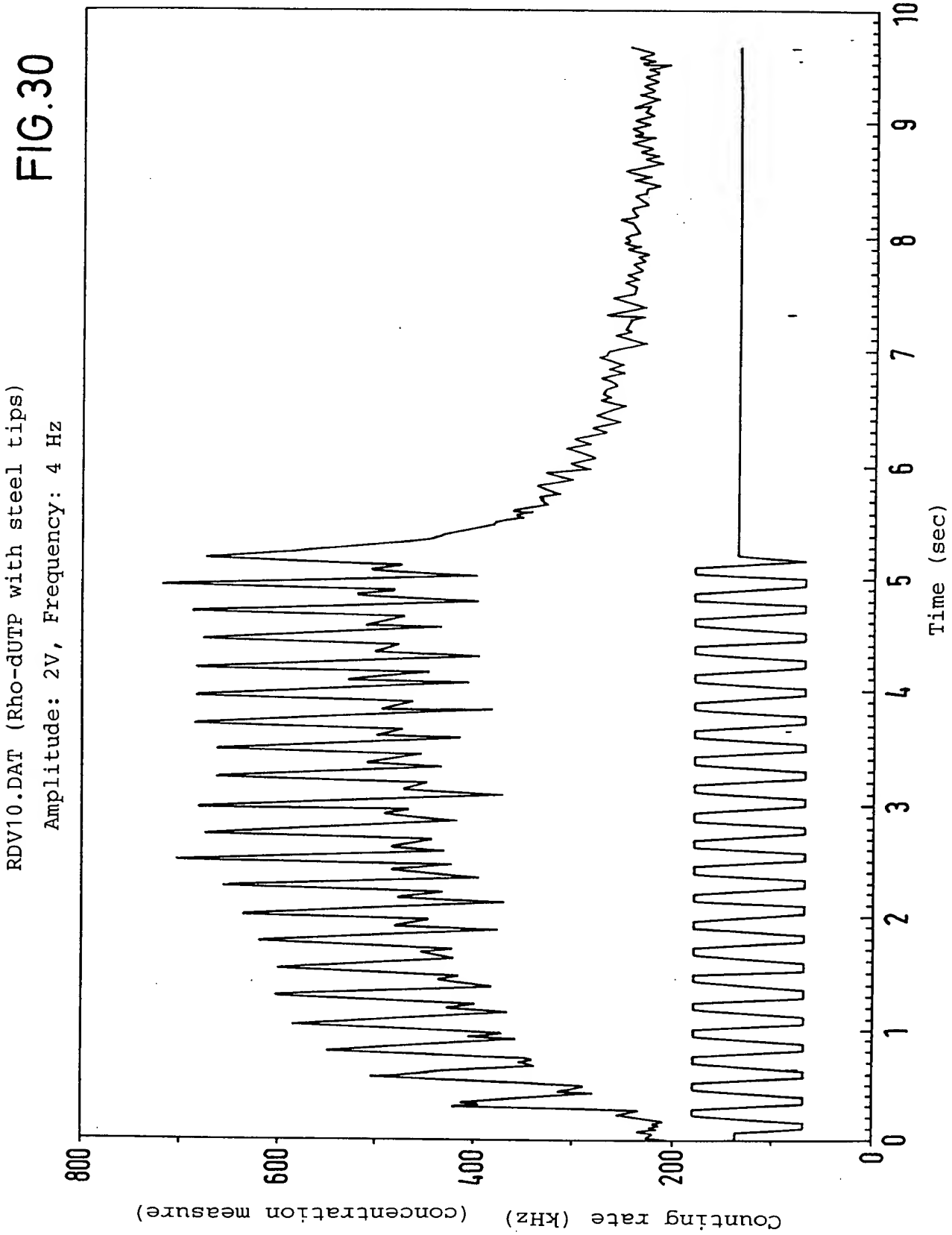

Bodipy Primer - M13 DNA Association
associated fraction
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
50
100
150
Time (min)

FIG.29

FIG.30


RDV10.DAT (Rho-dUTP with steel tips)
Amplitude: 2V, Frequency: 4 Hz
Counting rate (kHz)
(concentration measure)
800
600
400
200
0
0
1
2
3
4
5
6
7
8
9
10
Time (sec)

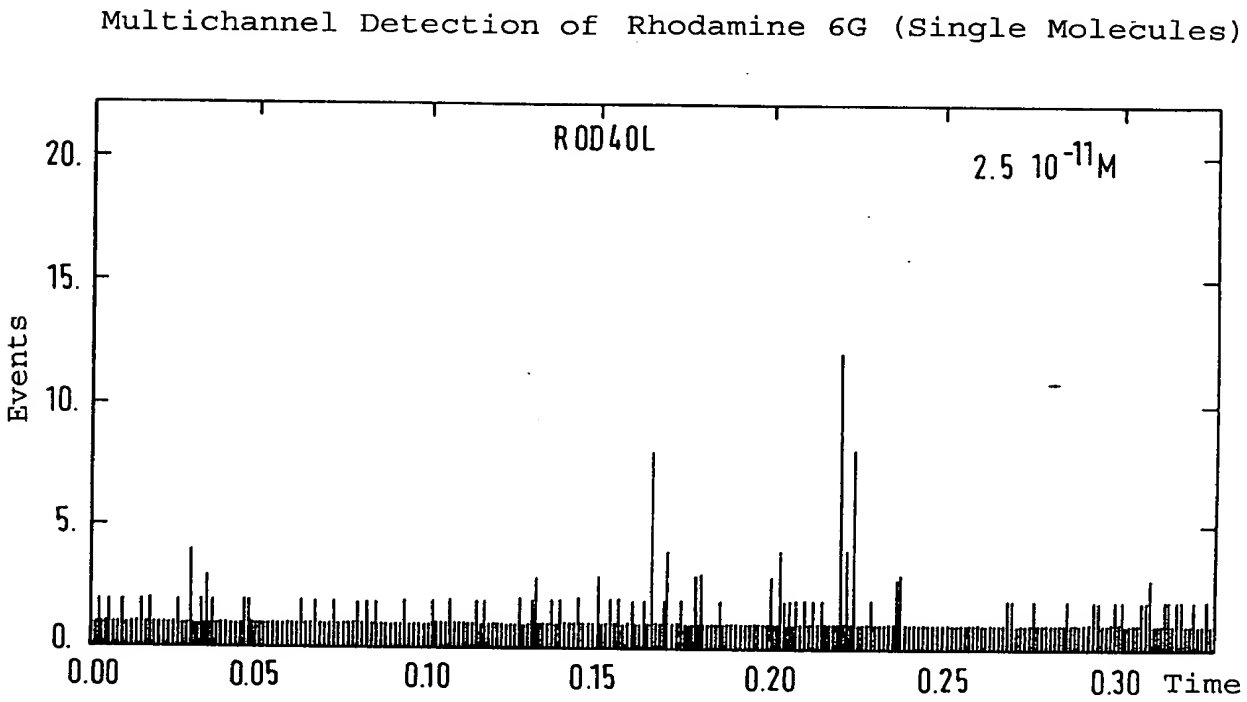


FIG.31a

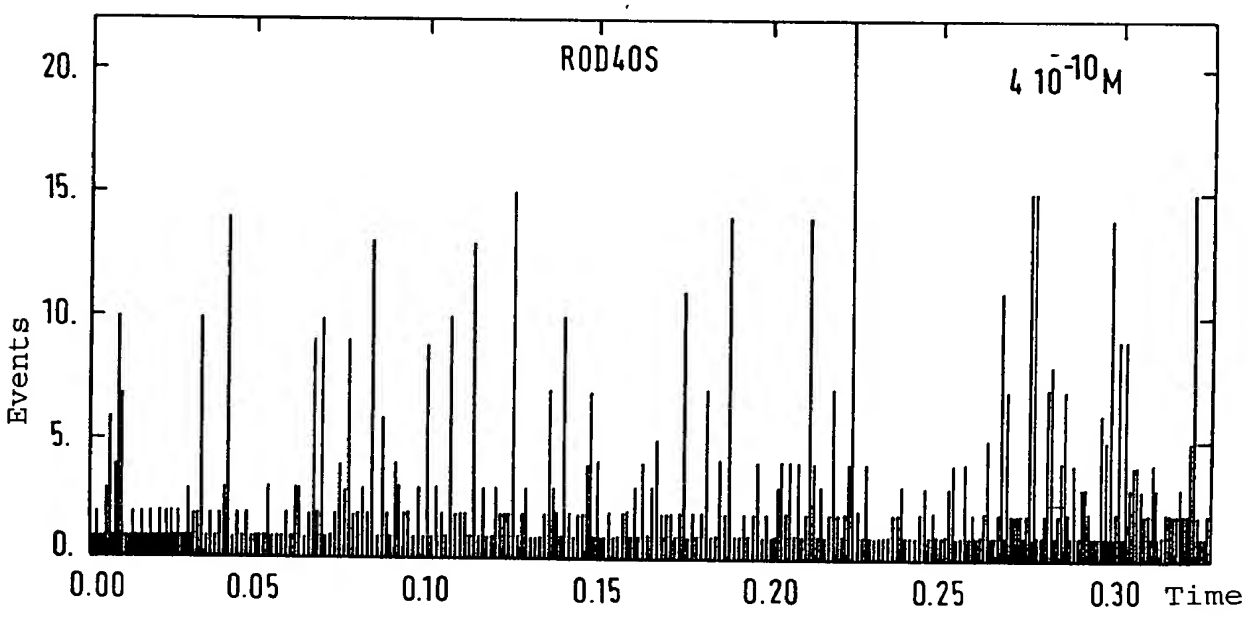


FIG.31b